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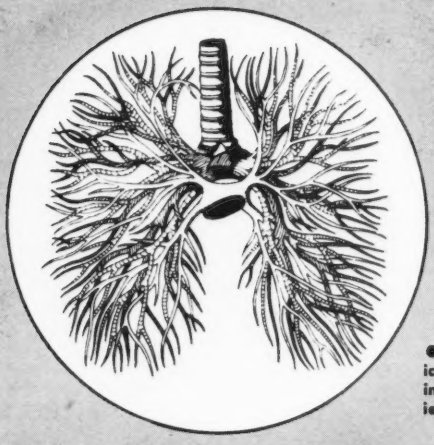

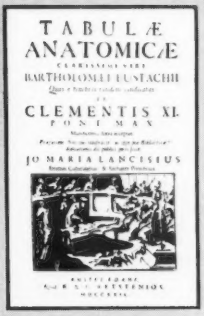

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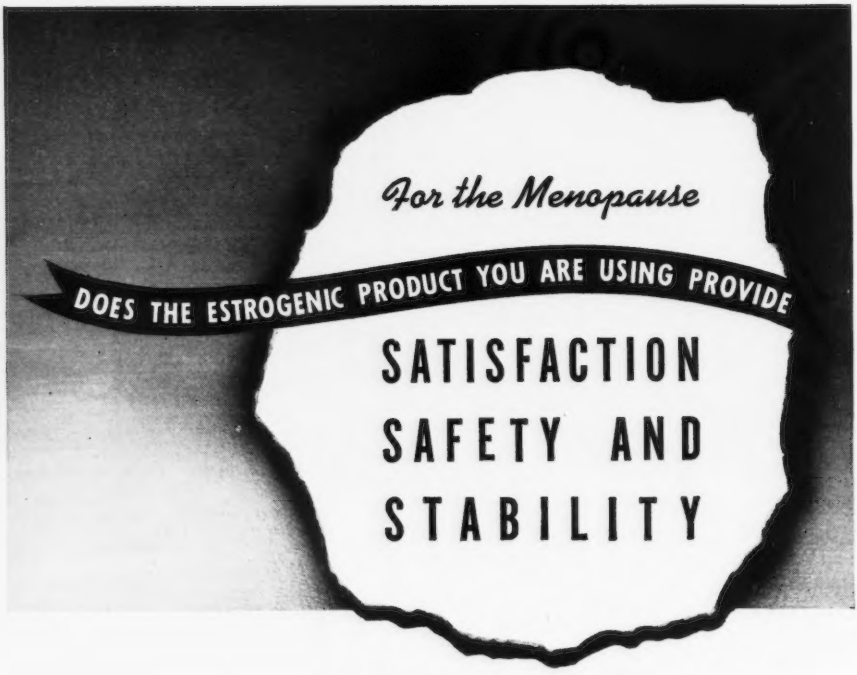

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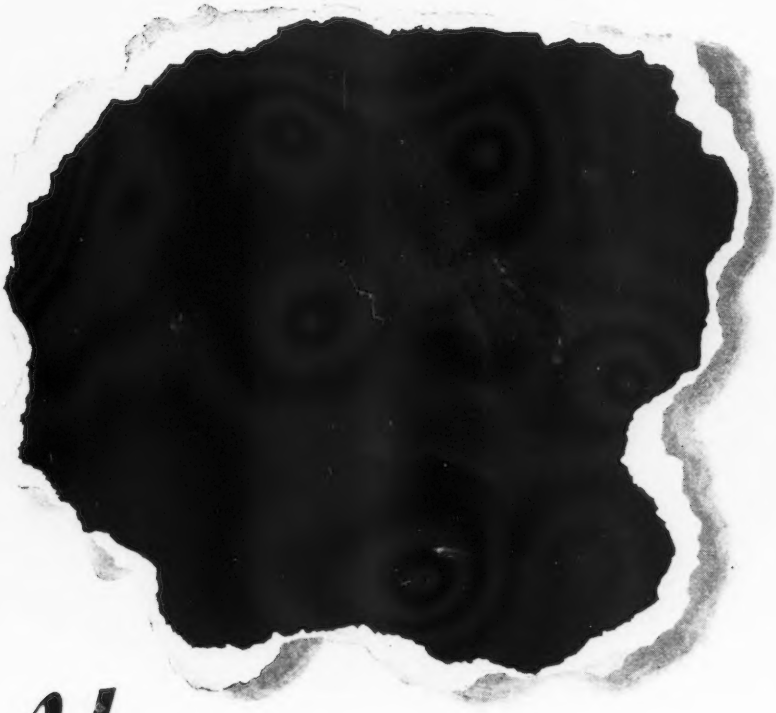

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VOLUME 32 JANUARY, 1943 NUMBER 1

STIMULATING EFFECT OF ADRENALECTOMY ON HAIR GROWTH AND MELANIN DEPOSITION IN BLACK RATS FED DIETS ADEQUATE AND DEFICIENT IN THE FILTRATE FACTORS OF VITAMIN B[1]

WITH A NOTE ON EFFECT OF DIET ON POST-OPERATIVE SURVIVAL

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NEW YORK, NEW YORK

IN VIEW of the reports that diets deficient in the filtrate factors of vitamin B have resulted in atrophy and necrosis of the adrenal cortex (1–4), as well as in graying of the fur in black or brown rats (5–7), we were prompted to study the effects of adrenalectomy in rats maintained on diets deficient in 'filtrate factors.' A reversal of the graying process, together with an acceleration of hair growth, followed ablation of both adrenal glands. This report is concerned with the investigation of the changes so far observed.

PROCEDURE

Rats of the Long-Evans strain bred in the laboratory, were used. The diet deficient in 'filtrate factors' consisted of casein 22 per cent, sucrose 64 per cent, primex 9 per cent, salt mixture 5 per cent, and 1.9 cc. of cod liver oil per 100 gm. of the mixture. In addition, the rats were given daily 15 micrograms each of thiamine chloride and pyridoxine and 45 micrograms of riboflavin. When the filtrate factors were added to the diet, they were given in the form of a rice bran concentrate,[2] 15 cc. per 100 grams. All rats were given a 1 per cent solution of sodium chloride ad libitum. The experiments were made in the fall, winter, and early spring seasons.

Four groups of adrenalectomized rats and 4 groups of unoperated rats on similar diets were studied (table 1). The first 2 groups consisted of animals maintained on the deficient diet prior to adrenalectomy. Following operation the rats in *group 1* were continued on the deficient diet and the rats in *group 2* were changed to the diet supplemented with the filtrate factors, referred to as the 'complete diet' in table 2. The third and fourth groups of rats were fed the supplemented diet before adrenalectomy. After operation the rats in *group 3* were changed to the deficient diet and

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[1] This research was aided by a grant from the Josiah Macy, Jr. Foundation.

[2] The rice bran concentrate (Galen B) was supplied by the Galen Company, Berkeley, Calif.

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TABLE 1. GROUPS OF RATS STUDIED

Group	Diet Before Adrenalectomy	Diet After Adrenalectomy	No. of Rats
1	Filtrate factor deficient	Filtrate factor deficient	43
2	Filtrate factor deficient	Supplemented with filtrate factor	15
3	Supplemented with filtrate factor	Filtrate factor deficient	6
4	Supplemented with filtrate factor	Supplemented with filtrate factor	6
	Control Rats		
	Preliminary diets	*Change in diets*	
1a	Filtrate factor deficient	Not changed	15
2a	Filtrate factor deficient	Supplemented with filtrate factor	10
3a	Supplemented with filtrate factor	Filtrate factor deficient	7
4a	Supplemented with filtrate factor	Not changed	6

the rats in *group 4* were continued on the complete diet. The control (unoperated) rats in all 4 groups were given the same diets. When the diets were changed for the operated rats, they were changed at the same time for the control animals. The completeness of adrenalectomy was checked by salt deprivation in animals surviving for long periods. In all animals, at death, the operative site and vicinity were carefully examined for accessory or residual bits of adrenal gland tissue. Histologic studies, including polariscopic examination for doubly refractive lipids and the application of silver nitrate solution for reducing substances, were made on doubtful tissues. When residual tissue was found the animal was discarded from the groups reported.

RESULTS

Appearance of the skin at the time of operation. At operation, an area about 1.5 inches wide across the back of the rat was shaved. It was observed that in rats maintained on the deficient diet, the skin was uniformly pink, whereas in rats on the

TABLE 2. EFFECT OF DIETS DEFICIENT AND ADEQUATE IN THE FILTRATE FACTORS ON SURVIVAL OF ADRENALECTOMIZED RATS

	No. of Rats in Group	Age at Operation, days	Weight at Operation, gm. Av.	Weight at Operation, gm. Range	Pre-op. Diet	Duration of Diet, Preop. days	Post-op. Diet	Survival, days Av.	Survival, days Range	Weight at Death gm. Av.	Weight at Death gm. Range	Pigmentation: Onset, days Av.	Pigmentation: Onset, days Range	Pigmentation: Pronounced, days Av.	Pigmentation: Pronounced, days Range
Diets deficient in filtrate factors before and after adrenalectomy															
Group 1	13	63	93	72–130	FFD	24	FFD	22.4	8–65	97	77–116	7	5–14	16	9–23
	5	62	108	95–124	FFD	28	FFD	24.6	14–47	107	95–115	6	6	13	13–14
	8	68	104	87–118	FFD	33	FFD	12.6	7–18	98	79–109	8	7–9	14	13–15
	5	71	109	78–130	FFD	36	FFD	13.6	7–20	105	76–121	6	6	16	14–17
	4	74	87	84– 94	FFD	38	FFD	12.0	5–22	89	80– 93	6	5–7	11	10–14
	4	88	120	116–123	FFD	45	FFD	20.5	15–27	111	109–114	5	5	16	15–16
	4	140	104	88–120	FFD	108	FFD	5.2	3–6	96	87–112	None			
Diet deficient in filtrate factors before adrenalectomy; complete diet after adrenalectomy															
Group 2	4	64	106	85–120	FFD	24	Complete	—	15–65[1]	129	100–157	6	5–8	12	11–13
	4	62	94	92– 97	FFD	28	Complete	18	13–26	108	98–114	6	6	13	13
	2	74	87	76– 98	FFD	38	Complete	24	14–23	109	88–130	5	5	14	14
	3	88	96	83–106	FFD	45	Complete	26	17–41	126	99–140	4	4	13	13
	2	110	80	71– 89	FFD	69	Complete	9.5	9–10	89	86– 91	8	7–9	None	
Complete diet before adrenalectomy and diet deficient in filtrate factors after adrenalectomy															
Group 3	5	78	184	137–228	Complete	36	FFD		43–60[1]	151	126–176	7	7	13	13
	1	50	136		Complete	18	FFD		96[1]	130		4		18	
Complete diet before and after adrenalectomy															
Group 4	5	78	139	130–148	Complete	36	Complete		43–61[1]	135	117–147	7	5–8	14	13–15
	1	50	114		Complete	18	Complete		98	159		5		15	

[1] Rats survived until deprived of salt.

complete diets (*groups 3, 4*) pigmented areas in band form were present on the sides or on the back. This led to a study of the distribution of such bands in normal rats. Animals from different litters were completely shaved and it was found that in the normal black rat pigmented bands of various widths occurred in one of several patterns. The bands extended either *a*), longitudinally from the neck to the hind legs in bilateral stripes, *b*), in a single broad stripe down the middle of the back, or *c*) in an X-shaped pattern across the back with a patch just above the tail. The normal shaved rats (fig. 1) illustrate one of the patterns of the 'pigmented bands' that was observed. In contrast, the rats in figure 2 had been on the diet deficient in the filtrate factors for 111 days and when shaved no sign of the pigmented bands was found. Histologic study has shown that the pigmented zones owe their color to the visibility through the overlying cutis of melanin accumulated in the massed hair bulbs and follicles in these areas. These studies are reported in detail in the section on histology.

When a normal young black or brown rat is placed on a diet deficient in the filtrate factor, graying of the fur occurs. The pattern of this graying is not the same in all rats and this apparently is due to the distribution of the pigmented areas. Graying appears first and is most marked in the areas of the pigmented stripes. As the graying proceeds, the pigmented bands disappear from the skin. The bands usually disappeared completely after the rats had been on the deficient diet for 24 days. The deficient diet therefore, not only causes depigmentation of the fur but also deprives the hair bulbs of their normal pigment.

Effect of adrenalectomy in rats deficient in filtrate factors. When rats on the deficient diet were adrenalectomized a striking change occurred in the appearance of the skin. This was first noted about 5 to 7 days after adrenalectomy. The skin developed a bluish color which deepened and reached its greatest intensity on the 14th to 16th day, and then gradually faded. By the 14th day new fur became visible and was either glossy black or brown, depending on the original color of the rat. The fur that regrew in the adrenalectomized rat presented a marked contrast to the fur that regrew in control rats on the deficient diet. In the adrenalectomized rat, the fur was abundant and deeply pigmented; in the control rats on deficient diet, the regrowth of fur following shaving was very sparse, very slow, and where it did regrow the color was dull gray. In shaved control rats that had been on the deficient diet for more than 30 days, the fur did not regrow at all. Figure 3 is that of 2 rats, an adrenalectomized rat deficient in the filtrate factors (*A8-12*) and an unoperated rat deficient in the filtrate factors (*A8-12a*). They were shaved on the same day. The photograph was taken 10 days after *rat A8-12* had been adrenalectomized, at which time both rats had been on the deficient diet for 48 days. The bluish color of the skin of the adrenalectomized rat on the deficient diet is typical of the change that was observed.

When rats, following adrenalectomy, were changed from deficient to complete diets (*group 2*), the same changes occurred in the appearance of the skin and fur (fig. 4, *rat A8–24*, adrenalectomized and changed to complete diet). The unoperated rats treated to a similar change in diet naturally showed the effects of the addition of the filtrate factors. Fur regrew gradually over the area shaved at the time the other rats were adrenalectomized, and as the deficiency was overcome the pigment bands reappeared. Rat *A8–24a* illustrates the change in the control rat occurring 17 days after the deficient diet had been supplemented with the filtrate factors. In the area shaved over the lower back to correspond to the shaved area in the operated rat, there was some coloration of the skin preceding the regrowth of fur. However, apart from the reappearance of the normal pigment bands, the only change in the color of the skin was in the shaved area. In the adrenalectomized rat, on the other hand, the

bluish color was visible over the entire body regardless of whether or not the fur had been shaved, as can be seen in the figure.

Effect of adrenalectomy on rats maintained preoperatively on diets supplemented with the filtrate factors. Following adrenalectomy in rats that had been fed the complete diet prior to operation (*groups* 3, 4), the same acceleration of hair growth and increased deposition of melanin in the hair follicles and bulbs occurred (fig. 5, 6). Some of the operated and control rats in both of these groups were shaved from the base of the tail to the back of the neck. In the unoperated rats in *group 3a*, changed from complete to deficient diets at the time their litter mates were adrenalectomized, the response to shaving reflected the effect of the deficient diet (fig. 5, *rat A11-13a*). The fur regrew slowly, there was no accumulation of pigment in the hair bulbs and the hair itself was gray. On the other hand, in the adrenalectomized rats in *group* 3, the absence of the filtrate factors in the diet post-operatively did not impair the increased deposition of melanin and the acceleration of hair growth (fig. 5, *rat A11-13*).

In the rats in *group* 4 that were adrenalectomized, identical changes in the hair-forming structures and in the deposition of melanin occurred. The changes appeared to be the most marked of any observed (fig. 6). The unoperated control rats regrew fur over the shaved area (fig. 6, *rat A11-23a*) but not as rapidly as the operated animals (fig. 6, *rat A11-23*). The regrowth of fur was better in the rats in this group than in any of the other control rats, as the diets were at no time deficient in the filtrate factors.

In none of the adrenalectomized rats thus far observed did the diffuse coloration

---

Fig. 1. NORMAL RATS on nu-chow diet, not adrenalectomized, age 94 days. The rats were shaved just prior to photographing. The skin shows one type of arrangement of the normal pigment bands.

Fig. 2. *Rats 260-1* and *270-1*. The diet had been deficient in the filtrate factors for 111 days, at which time the animals were shaved and photographed. The entire skin was pink and no pigment bands were found. Age, 147 days.

Fig. 3. *Rat A8-12*, ADRENALECTOMIZED, FILTRATE-FACTOR DEFICIENT; 10 days post-adrenalectomy and Rat *A8-12a*, UNOPERATED, FILTRATE-FACTOR DEFICIENT. Both rats were given the deficient diet when 35 days old. On the 39th day of the diet *rat A8-12* was shaved across the back and adrenalectomized. The unoperated control rat was shaved across the back on the same day. Ten days post-adrenalectomy, *rat A8-12* died. The area over the right shoulder was shaved at death. This photograph was taken immediately after death. *Rat A8-12a* was killed on the same day and the area over the right shoulder was shaved. Note the diffuse bluish color of the skin of the adrenalectomized rat as compared to the pinkish color of the skin in the unoperated rat. Total period of the deficient diet in both rats was 49 days. Age at death, both rats, 84 days.

Fig. 4. *Rat A8-24*, 17 days post-adrenalectomy. THIS RAT was on the diet deficient in the filtrate factors for 39 days prior to adrenalectomy. After operation the rat was placed on the complete diet. *Rat A8-24a* is the control. This animal was shaved across the lower back on the day that *A8-24* was adrenalectomized and was also changed to the complete diet on this day. Seventeen days later both rats were shaved over the left shoulder and back, killed and photographed. In the adrenalectomized rat there is the characteristic bluish black color over the entire back and regrowth of glossy black fur over the area shaved at operation. The control rat shows slight regrowth of fur over the shaved area and a return of the pigment bands.

Fig. 5. *Rat A11-13*, 16 days post-adrenalectomy; complete diet for 18 days; changed to diet deficient in filtrate factors after adrenalectomy. *Rat A11-13a*, unoperated rat; same age; complete diet for 18 days, then changed to diet deficient in filtrate factors. *Rats A11-13* and *A11-13a* were begun on the complete diet when 32 days old. After 18 days on the diet both rats were shaved over the entire back and *rat A11-13* was adrenalectomized. After adrenalectomy both rats were changed to the diet deficient in the filtrate factors. This photograph was taken 16 days after shaving and adrenalectomy. Note the re-growth of black fur in the operated rat and the fading of the pigment bands in the unoperated rat.

Fig. 6. *Rat A11-23*, adrenalectomized rat; 18 days post-adrenalectomy; complete diet for 36 days. *Rat A11-23a*, unoperated rat; same age; complete diet for 36 days. *Rats A11-23* and *A11-23a* were begun on the complete diet when 32 days old. After 18 days on the diet both rats were shaved over the entire back and *rat A11-23* was adrenalectomized. The rats were continued on the complete diet. This photograph was taken 18 days after shaving and adrenalectomy. Note the regrowth of thick black fur on the adrenalectomized rat and the moderate regrowth of fur in the unoperated rat. The regrowth of fur in the unoperated rat is most marked in the areas of the original pigment bands.

1.

Normal Rats

2.

Filtrate-Factor Deficient

3.

Adrenalectomized A8-12 Unoperated A8-12a

4.

Adrenalectomized A8-24 Unoperated A8-24a

5.

Adrenalectomized A11-13 Unoperated A11-13a

6.

Adrenalectomized A11-23 Unoperated A11-23a

of the skin persist. Biopsies of the skin made 1 to 2 months after adrenalectomy showed that the hair bulbs had reverted towards a resting state.

Histological Observations

Normal rats. For histological comparison with the skins of rats deficient in filtrate factors and adrenalectomized rats, normal rats were killed at intervals varying from 10 to 160 days of age. The normal rats were maintained on the nu-chow diet. Blocks of skin, including subcutaneous tissue and superficial muscle, were chosen from both pink and pigmented areas. The latter have been referred to as pigmented stripes or bands.

In the young rats, up to about 4 weeks of age, the entire dorsal skin appeared bluish black. On histological examination no differences were found in samples from various portions of the dorsal skin, the hair bulbs and follicles being closely spaced, numerous and actively growing. After 30 to 35 days, most of the dorsal skin had lost the bluish-black color and appeared pink on shaving. As mentioned previously, pigmented stripes persisted in various patterns. Sections through the bands showed pigmented hair bulbs in greater number than in the pink areas of skin (fig. 7, 8). There was no pigment present in the cutis itself in either zone. The pigmented bands seen on shaving owe their color to the melanin in the massed hair bulbs and follicles. The hair bulbs in the pigmented bands are usually in an active state, with cellular roots and much melanin in the papilla and the hair shaft. In the hairs themselves, granular or homogeneous masses of pigment were distributed throughout the shaft of the hair at regular intervals.

As is well known, the hairs in any given section of skin in an adult rat may be in various stages of growth, disintegration or replacement. The chief difference between the pigmented and pink zones in the rat's skin lies in the greater number of hairs and, therefore, the more active hair growth in the pigmented zone. Furthermore, the hair bulbs in the pigmented zones were more deeply placed and the hairs were mostly of the coarse terminal type. The hairs in the pink zones were chiefly of the thin lanugo type.

Confirming the impression of the active deposition of melanin was the fact that the 'dopa oxidase' reaction, method of Laidlaw (8), was absent from the skin of the pink zones but was strongly positive in the active hair bulbs in the pigmented stripes.

Rats deficient in filtrate factors. The graying of the hair in black or brown rats that results from filtrate-factor deficiency is associated with atrophy of the hair apparatus and a cessation of melanin deposition. As a result of the atrophy, hair growth becomes extremely slow and shaving does not act as a stimulus to the process. The hairs themselves are brittle and tend to fall out. The pigmented stripes seen in the shaved normal rat disappear if rats are fed the deficient diet for 20 to 28 days. In cross sections of skin of the dorsal area no deeply placed bulbs were found and the hair follicles were more widely spaced (fig. 9, 10). The spacing was similar to that seen in the pink skin from older rats on a normal diet. The disappearance of pigment from the hair follicles was confirmed repeatedly and dopa tests on frozen sections of unfixed skin revealed the absence of 'dopa oxidase' in the hair apparatus. In a single series of biopsies in rats fed the deficient diet, the 'dopa oxidase' disappeared in the fourth week.

Influence of adrenalectomy. Adrenalectomy resulted in similar changes in the skins of the normal rats and those deficient in filtrate factors. The changes were more striking in the deficient rats because the atrophic state of the hair follicles was reversed (fig. 11, 12). For brevity, the effects in both groups have been considered together. Biopsies taken 3 to 5 days (fig. 11) after adrenalectomy showed marked

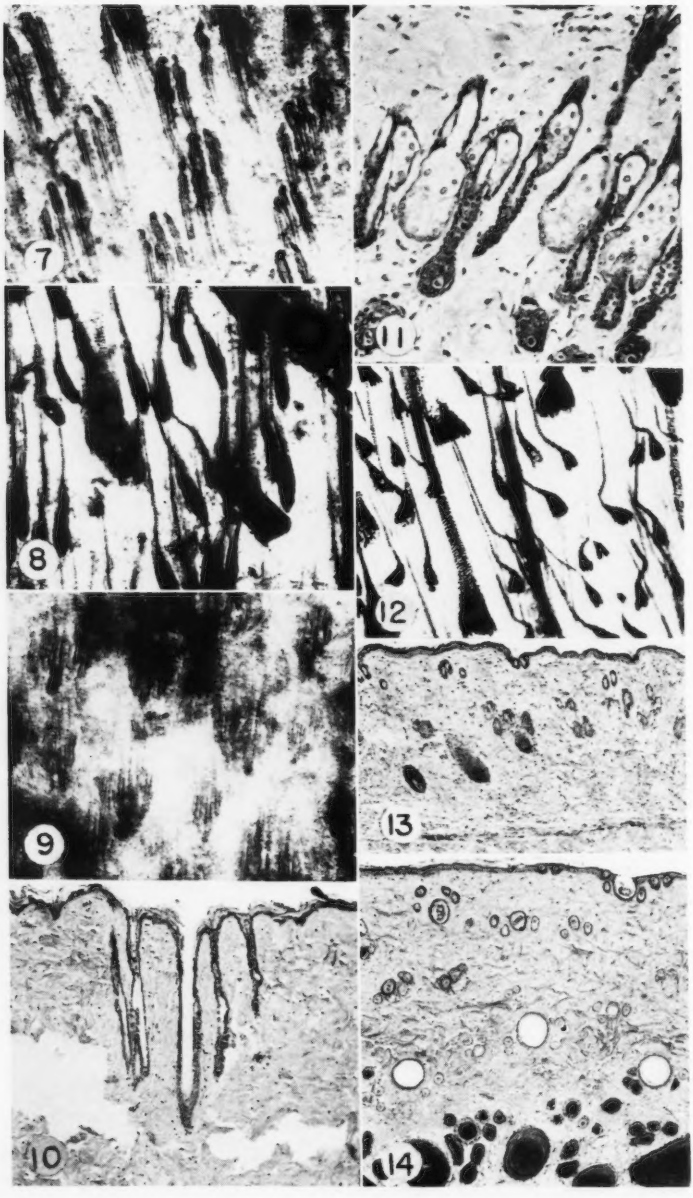

Fig. 7, 8. DORSAL, PINK SKIN AND PIGMENTED BAND OF SKIN RESPECTIVELY; normal rat 110 days old. Pink skin stained with iron hematoxylin and dehydrated. Skin from pigmented band unstained. Note the pattern of distribution of the hair follicles and bulbs in clusters. In the normal rat only the most central and most deeply placed bulbs regularly show active melanin deposition. This is in contrast to the state of the bulbs in the pigmented bands, all of which show active melanin deposition. ×40.

stimulation of the hair bulbs in both the normal and atrophic skin. As early as 3 days after operation long strands of epithelial cells were found extending downward from pre-existing hair bulbs. Mitoses were numerous in the cell strands. New bulbs formed rapidly from neighboring hairs until the peak of hyperplasia was reached 14 to 16 days after adrenalectomy (fig. 12, 13, 14). During this period there was also a marked increase of deposition of melanin in the new hair bulbs and follicles. The blue appearance of the skin resulted from the visibility of the melanin through the overlying cutis. No pigmentation occurred in the cutis itself.

The hyperplastic state persisted up to 30 days and then the process began to subside. However, large numbers of hyperplastic bulbs and follicles were found as late as 60 days after operation.

Observations on the deficient rats could not be very prolonged as only an occasional animal survived adrenalectomy for more than 22 days. The impression formed from the limited observations was that the increased hair growth and melanin deposition was of shorter duration in the rats on the deficient diet than in rats maintained on the complete diet. Inasmuch as the hairs of the deficient rats were gray and free from melanin at the time of adrenalectomy, the increased melanogenesis after operation was naturally more striking than in the animals on the complete diet.

Although the beginning hyperplasia of hair follicles was apparent by the 3rd post-operative day, the increased melanin deposition was not apparent until the 5th day. At this time progressively larger quantities of melanin granules were formed in the center of each new papilla and hair shaft. The result was that, as they grew, the new hairs had dense masses of melanin arranged in segments. Dopa tests confirmed the lag between the epithelial proliferations of the hair bulbs and the appearance of melanin. 'Dopa oxidase' was absent until the 5th to 7th post-operative day. As the melanin production increased there was an accompanying increase in 'dopa oxidase,' until the 28th to 30th day. Iron stains showed no iron-containing pigment in the proliferating hair bulbs or follicles.

Influence of Diet Deficient in Filtrate Factors on Survival

Survival following adrenalectomy appeared to be definitely influenced by the length of time the animal was on the diet deficient in the filtrate factors before operation (table 2). Rats continued on the deficient diet post-operatively did not long survive adrenalectomy. The survival depended principally on the duration of the deficient diet pre-operatively but was also somewhat influenced by the weight of the rat at the time of operation. Rats maintained on the deficient diet from 24 to 28 days

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Fig. 9. DORSAL SKIN from rat on diet deficient in filtrate factors for 65 days. The pattern is about the same as in the normal rat, but there is no melanin deposition in the bulbs or in the hairs. Iron hematoxylin (×40).

Fig. 10. DORSAL SKIN from rat on diet deficient in filtrate factors for 57 days. The wide spacing and resting state of the hair bulbs is shown. The root of a hair is included and shows the absence of melanin normally present in the hair of this species (×60).

Fig. 11. DORSAL SKIN of rat on the diet deficient in filtrate factor 3 days after adrenalectomy. Down growth of epithelial strands attended by mitotic proliferation is shown (×60).

Fig. 12. DORSAL SKIN from rat on diet deficient in the filtrate factor 18 days after adrenalectomy. The rat had been on the deficient diet for a total of 62 days. The hyperplastic bulbs exhibiting active deposition of melanin are crowded and are both superficial and deeply placed (×40).

Fig. 13, 14. DORSAL SKIN of rats 7 and 16 days after adrenalectomy, respectively. The rats had been fed diets supplemented with the filtrate factors both before and after adrenalectomy. Differences in the shape of the bulbs are due to the plane of the section and to notable increases in the size of the bulbs themselves (×40).

NOTE: Figs. 7, 8, 9 and 12 are of pieces of skin photographed after clearing. The other photomicrographs are of paraffin sections.

prior to adrenalectomy survived for an average of 22.4 and 24.6 days. Rats maintained on the diet 36 and 38 days prior to adrenalectomy survived for an average of 13 and 12 days, respectively. However, one group of 4 rats on the deficient diet 45 days before adrenalectomy survived 20.5 days; their average weight was 119.7 gm., which was 20 to 30 gm. heavier than the average weight of the other groups of rats. When the period of the dietary deficiency prior to operation was extended to 106 or 110 days, the rats survived adrenalectomy only 3 to 6 days.

The post-operative survival of rats restored to a complete diet after adrenalectomy was somewhat longer than that of rats continued on the deficient diet. However the duration of maintenance on the deficient diet pre-operatively again influenced the survival time (table 2). For example, 2 adrenalectomized rats that had been on the deficient diet for 24 days before operation survived as long as 62 days, at which time they were deprived of salt and died 3 days later. The other rats in this group died spontaneously on the 15th and 47th days. When the period of dietary deficiency pre-operatively was increased to 30 days or more, even though the rats were fed the complete diet after adrenalectomy, survival was strikingly reduced (table 2).

Although a sufficiently large number of rats have not been studied to establish an absolute relationship between the duration of the deficient diet pre-operatively and the post-operative survival when the animals were given a complete diet, it seems clear that animals subjected to filtrate-factor deficiency prior to adrenalectomy do not survive the operation as long as rats given adequate amounts of the filtrate factor before operation. This is borne out by the results in rats maintained on the complete diet prior to operation and changed to the deficient diet afterwards (*group* 3, table 2). All rats in this group survived until salt was withdrawn, after 41, 58, and 94 days. The same was true of rats kept on the complete diet both before and after adrenalectomy (*group* 4). All but one of the rats in *groups* 3 and 4 survived until deprived of salt which was done at periods varying from 41 to 94 days.

DISCUSSION

The observations reported confirm again the fact that diets deficient in the filtrate factors of vitamin B result in graying of the fur in black or brown rats. In addition, several interesting facts concerning the graying were brought out. The morphological basis of the graying was found to be atrophy of the hair follicles and bulbs and cessation of melanin deposition. The cause of the different patterns of graying that may occur was found to depend on the distribution of the pigmented bands normally present in the skin of rats.

When rats on diets deficient in filtrate factors were adrenalectomized, the atrophy resulting from filtrate-factor deficiency was not only arrested, but a pronounced stimulus to growth and hyperplasia of the hair follicles and bulbs was provided. Accompanying this there was a striking increase in the deposition of melanin in the hair bulbs and follicles. The stimulation to the growth of fur that occurred was similar to that reported by Butcher in white rats after adrenalectomy (9, 10). The difference between the black and the white rats following adrenalectomy is due to the presence of pigment in the former. It is the accumulation of melanin pigment in the proliferating hair bulbs of the black rat that becomes visible through the thin overlying cutis and accounts for the bluish appearance of the skin.

The changes observed have not been previously recorded, probably because adrenalecomy in rats has been performed for the most part in white rats. Many other species of animals have been adrenalectomized, e.g. cats, opossums, squirrels, groundhogs and dogs (11), but there have been few observations bearing on our findings. Harrop and Weinstein (12) noted 'pigment freckles' over the back and chest wall and

a slightly increased pigmentation of the lips in one monkey. For the most part, as Grollman has remarked (13), the pigmentation characteristic of adrenal cortical insufficiency in man has not been observed in experimental adrenal insufficiency. Whether or not the increase in melanin in the hair apparatus that occurred in the rats in our experiments is analogous to the pigmentation seen in human adrenal cortical insufficiency cannot be determined on the basis of the present evidence. We found no abnormal deposits of pigment in the mucous membranes of the mouth or intestine or in the cutis itself. Species variation and time factors prevent strict comparison of the pigmentation of experimental and clinical adrenal insufficiency. The findings in these experiments do, however, establish the fact that in the absence of the adrenal glands there is a stimulation to the deposition of melanin. Since the hair apparatus is a part of the ectodermal derivatives, it seems reasonable to include the effect observed in the rats among the cutaneous changes already recognized as part of adrenal insufficiency. The accleration of hair growth after adrenalectomy, reported earlier by Butcher (9, 10), has received little attention. No counterpart has been reported in human beings suffering from adrenal insufficiency. This may be due to the fact that the entire adrenal gland is removed in the rats. Furthermore, the experimental production of adrenal insufficiency is abrupt. In human beings adrenal cortical insufficiency is a chronic process and although eventually the entire gland may be involved, the disease apparently begins in the cortex itself.

SUMMARY

Seventy black or brown rats were adrenalectomized. Forty-three of the animals were on diets deficient in the filtrate factors of vitamin B for varying periods before and after operation. Fifteen rats were on the deficient diet pre-operatively and were changed to diets supplemented with the filtrate factors after operation. The remaining 12 rats were on complete diets pre-operatively and 6 were changed to deficient diets after operation. A group of control rats were treated in a similar manner.

The rats maintained on the diets deficient in filtrate factors pre-operatively showed varying degrees of graying of the fur, depending on the duration of the diet. When shaved, their skins were uniformly pink. The rats fed the complete diet were found, on shaving, to have pigmented bands in the skin arranged in one of several patterns. The bands were due to the accumulation of melanin in the massed hair bulbs and follicles. Similar bands were found in the skins of the stock animals and are characteristic of black or brown rats. When black rats are fed diets deficient in the filtrate factors, atrophy of the hair bulbs and follicles and cessation of melanin deposition occurs. This accounts for the graying of the fur, characteristic of the deficiency. The graying occurs first and is most pronounced in the areas of the pigmented bands. As the bands have different patterns, the pattern of graying is likewise different.

Adrenalectomy is accompanied by a marked transitory hyperplasia of the hair bulbs and follicles with an increased deposition of melanin. The stimulus provided is great enough to overcome the atrophy resulting from the filtrate-factor deficiency. Owing to the visibility of the concentrated melanin in the hair bulbs through the thin overlying cutis, the skin of the rat appears bluish. The color was evident about 5 to 7 days after adrenalectomy and was present over the entire body, regardless of shaving. The only area excepted was the ventral surface of those animals in which the fur had been white originally. The hyperplasia of the hair apparatus and the increased deposition of melanin increased in intensity, reaching a peak on approximately the 16th day after adrenalectomy. The fur began to grow out about the 14th day and the regrown fur was a deep glossy black or brown. The hyperplastic state, as observed on repeated histological examinations, persisted for about 30 days and then

the process began to subside. The active deposition of melanin was confirmed by the fact that the 'dopa oxidase' reaction, which was absent from the skins of the unoperated rats on deficient diets was present in the adrenalectomized rats on deficient diets.

The duration of maintenance on the deficient diet pre-operatively influenced the survival time after adrenalectomy. Animals maintained on the deficient diet before and after operation survived for an average of only 18 days. As the period of the deficient diet pre-operatively increased, the survival time decreased. The weights of the rats at the time of operation modified the survival time somewhat. Supplementing the diet with the filtrate factors after adrenalectomy increased the survival time somewhat. Rats which were on the complete diet before adrenalectomy, even though changed to the deficient diet after operation, survived, unless deprived of salt, as long as 96 days.

We are indebted to Miss Amy Fortune and Mrs. Henryka Berger for their technical assistance in this work, and to Mr. Edgar Nabel for the photography.

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REFERENCES

1. Morgan, A. F., and H. D. Simms: *Science* 89: 565. 1939.
2. Daft, F. S., and W. H. Sebrell: *Pub. Health Rep.* 54: 2247. 1939.
3. Nelson, A. A.: *Pub. Health Rep.* 54: 2250. 1939.
4. Ashburn, L. R.: *Pub. Health Rep.* 55: 1337. 1940.
5. Morgan, A. F., and H. D. Simms: *J. Nutrition* 19: 233. 1940.
6. Morgan, A. F., B. B. Cook and H. G. Davison: *J. Nutrition* 15: 27. 1938.
7. Unna, K., C. V. Richards and W. L. Sampson: *J. Nutrition* 22: 553. 1941.
8. Laidlaw, G. F., and S. N. Blackberg: *Am. J. Path.* 8: 491. 1932.
9. Butcher, E. O., and R. A. Richards: *Endocrinology* 25: 787. 1939.
10. Butcher, E. O.: *Proc. Soc. Exper. Biol. & Med.* 48: 120. 1941.
11. Britton, S. W.: *Am. J. Physiol.* 32: 9, 99. 1931.
12. Harrop, G. H., and A. Weinstein: *J. Exp. Med.* 57: 305. 1933.
13. Grollman, A.: The Adrenals. Williams & Wilkins Co., Baltimore, 1936. pp. 172.

BIOASSAY OF THE PITUITARY GROWTH HORMONE. WIDTH OF THE PROXIMAL EPIPHYSIAL CARTILAGE OF THE TIBIA IN HYPOPHYSECTOMIZED RATS[1,2]

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HYPOPHYSECTOMY causes regressive changes at the proximal end of the tibia in the immature rat, as described by various authors (1–4). Within a few days, the thickness of the cartilage plate decreases significantly, a consequence of a retardation or cessation of cartilage formation, and of an active destruction of cartilage. Administration of pituitary growth hormone reverses these regressive changes (1, 2, 4, 5). The initial effects of growth-hormone treatment consist predominantly of chondrogenesis. Activation of osteogenesis follows later. The width of the cartilage disc increases during the first 6 to 8 days of treatment with growth hormone until the normal equilibrium between chondrogenesis and osteogenesis is reestablished. During this initial period of administration of growth hormone the resulting increase in width of the cartilage is, within certain limits of dosage, proportional to the quantity of growth hormone injected (5).

Based on these phenomena, a new method was developed for the bioassay of the pituitary growth hormone. In order to devise a simple and rapid procedure, practical for routine work, it was decided to avoid time-consuming techniques such as preparation of serial sections of bone and complex staining methods. After some preliminary work, in which experimental conditions had been varied, the following procedure was adopted as standard for growth hormone assay.

METHOD

Immature female rats are hypophysectomized when 26 days old. After a postoperative interval of 12 days, the growth hormone preparation of unknown potency which is dissolved in saline, is injected intraperitoneally; daily administration is continued for a total of 4 days. Twenty-four hours after the last injection the animals are autopsied, the right tibias taken, freed from soft tissues, split at the proximal end in a sagittal plane, and fixed in neutralized 10 per cent formalin, or stained immediately. Previous to staining, the bone halves are washed thoroughly, immersed in acetone for at least one hour and washed again. They are then immersed for about 1½ minutes in 2 per cent silver nitrate solution (6) and exposed to a strong light, while kept under water, until calcified parts appear dark brown. They are then fixed in a 10 per cent solution of sodium thiosulfate for about one-half of a minute, followed by thorough washing under running water.

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[2] Aided by grants from the Research Board of the University of California and from the National Research Council Committee on Research in Endocrinology.

The appearance of the stained surface of the proximal end of the longitudinally split tibia is indicated in figure 1. While calcified tissue is stained dark brown, the proliferating zone of the cartilage appears as a clearly defined white band whose thickness can easily be measured under a microscope with a calibrated micrometer eyepiece. For routine determinations, at least 8 individual readings are made across the width of the epiphysis.

In figure 1 the tibia of a control rat is compared with that of an animal injected daily for 4 days with 5 growth-hormone units (hypophysectomized rat units, body weight tests, reference 7). It is obvious that the increase in width of the proliferating zone of the cartilage after treatment with growth hormone is highly significant. In order to study the relationship between dosage and response, a number of different dilutions was prepared of a standard pituitary growth hormone preparation. The latter was a 'cysteine-treated globulin fraction,' made from beef anterior pituitary

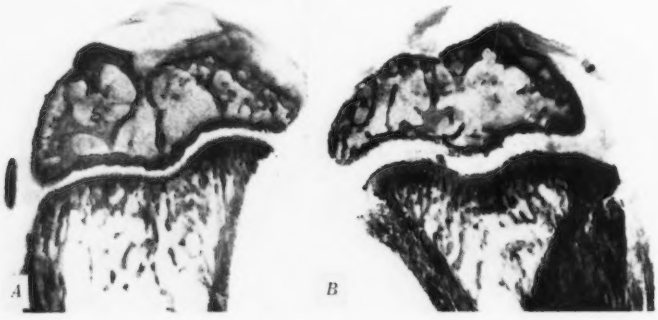

Fig. 1. PROXIMAL ENDS OF TIBIAS longitudinally split and stained by silver nitrate, from hypophysectomized rats. A. Untreated control. B. Treated with growth hormone.

according to a purification procedure described elsewhere (8). It contained approximately 25 growth hormone units per milligram (hypophysectomized rat units, body weight test, reference 7). No follicle-stimulating or interstitial-cell-stimulating hormones were demonstrable when this preparation was given to hypophysectomized rats at a total dose of 5.0 mg. (4-day test), but there were traces of thyrotropic and adrenocorticotropic activities. In such preparations, contamination with lactogenic hormone was always found to be below 0.5 per cent. A total of 173 hypophysectomized rats was used. Each dosage level was given to a group of 8 to 25 animals. When the resulting average width of the proliferating zone of the cartilage was plotted as the ordinate, expressed in micrometer divisions, against the logarithm of the daily dose as abscissa, a straight line resulted within certain dosage limits as indicated in figure 2, *A*. For comparison, the body weight gain of the same group of rats, plotted against the logarithm of the dose, is shown in figure 2, *B*. It is obvious that, in a test period of 4 days, variations are much larger for the body weight increase than for the response of the epiphysial cartilage.

In order to determine the accuracy of the method, standard errors were determined for the experimental groups. The mean error was found to be $\sigma = 0.42$ micrometer scale divisions. A difference in response of at least 3 times this value, 1.26 scale divisions, was considered statistically significant. From the diagram shown in figure 2, *A*, it can be calculated that for a given dose, a change of at least 100 per cent is required to cause a significant change in response.

Although it was found that a value of 1.26 scale divisions represented a statistically significant difference, it was decided to choose, arbitrarily, twice this value as the basis for the minimal significant response. Therefore, the minimal effective dose of

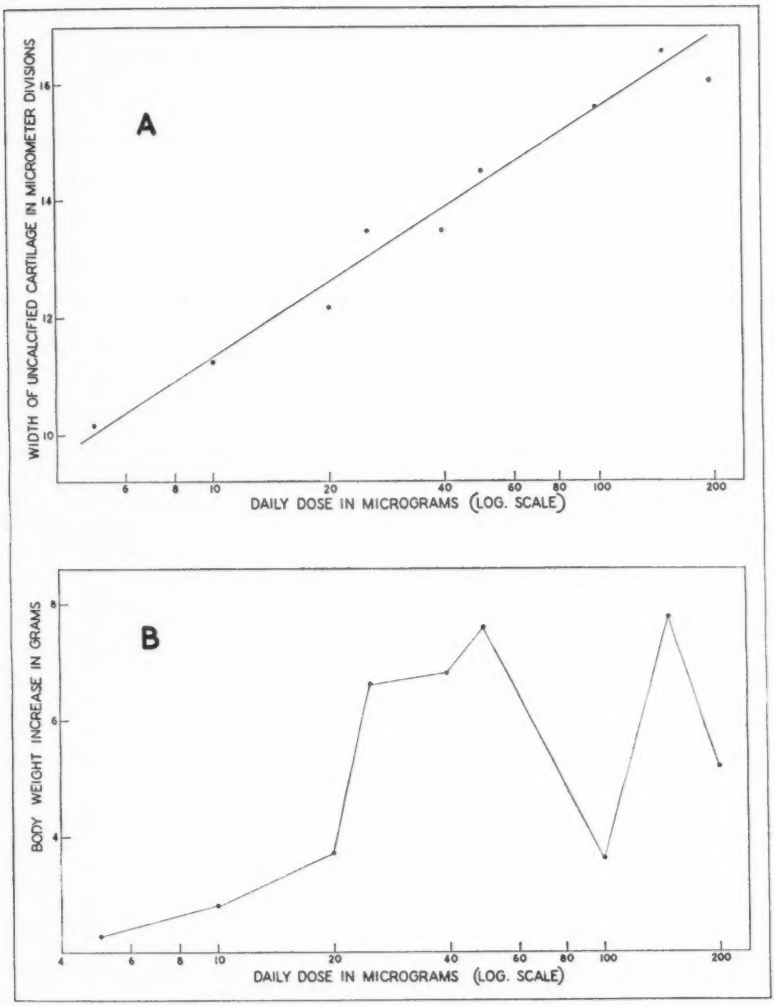

Fig. 2. Response of hypophysectomized rats to growth hormone. 4-day test. *A*. Width of uncalcified epiphysial cartilage of tibia. *B*. Increase in body weight.

a growth hormone preparation was defined as the amount given under the experimental conditions described above which had caused an increase in width of the proliferating zone of the cartilage over the control value of 2.5 scale divisions. One growth hormone unit was defined for this test method as the equivalent of the quantity of growth hormone causing this same effect. For the growth hormone preparation used

in the present work, the daily minimal effective dose so defined was found to be 12 micrograms.

Comparisons of the accuracy and sensitivity of the new bone test and the earlier method based on body weight for growth hormone assay are shown in table 1. It is obvious that approximately the same accuracy was obtained in an injection period of 4 days with the new test, as was obtained with an experimental period of 15 days. The new bone test was found to be approximately 3 times more sensitive, calculated on the basis of the daily dosage. The quantity of hormone required for the total

TABLE 1. COMPARISON OF BONE TEST AND BODY WEIGHT METHOD FOR GROWTH HORMONE ASSAY

Method	Injection Period in Days	Accuracy	Sensitivity	
		Minimal dose level multiples necessary to produce significant changes in response	Micrograms of growth hormone preparation required for minimal effective dose	
			per day	for total test period
Bone test	4	2.0	12	48
Body weight test	15	2.1	40	520

minimal effective dose was, in the new test, about 1/11 of what had been necessary for the body weight method.

Until the response of the epiphysial disc to other hormones, such as pituitary thyrotropic hormone, thyroxin, pituitary lactogenic hormone and insulin has been more carefully studied, it can not be said that this test is more specific than the body weight increment method. It should be stressed, however, that the purest growth hormone preparations so far made by us have given this response at low doses.

SUMMARY

A method is described for quantitative determination of the pituitary growth hormone in the hypophysectomized rat, using the width of the uncalcified cartilage of the proximal epiphysis of the rat's tibia as the criterion. The relation between the logarithm of the dosage and the response of the cartilage could be represented, within a limited range of doses, by a straight line. The accuracy of the method was determined and a growth hormone unit was defined. The bone test is approximately 3 times as sensitive as the body weight method (on the basis of the daily dose), and requires 4/15 of the time and about 1/11 of the quantity of hormone necessary for a corresponding response in the body weight method.

REFERENCES

1. FREUD, J., L. H. LEVIE AND D. B. KROON: *J. of Endocrinology* 1: 56. 1939.
2. RAY, R. D., H. M. EVANS AND H. BECKS: *Am. J. Path.* 17: 509. 1941.
3. INGALLS, T. H., AND D. R. HAYES: *Endocrinology* 29: 270. 1941.
4. BECKS, H., E. A. KIBRICK, W. MARX AND H. M. EVANS: *Growth* 5: 449. 1941.
5. KIBRICK, E. A., H. BECKS, W. MARX AND H. M. EVANS: Growth 5: 437. 1941.
6. VON KOSSA, J.: *Beitr. z. path. Anat. u. z. allg. Path.* 29: 163. 1901.
7. MARX, W., M. E. SIMPSON AND H. M. EVANS: *Endocrinology* 30: 1. 1942.
8. MARX, W., M. E. SIMPSON AND H. M. EVANS. In press.

FIRST RESPONSE OF DEVELOPING OPOSSUM GONADS TO EQUINE GONADOTROPIC TREATMENT[1]

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THE QUESTION of the degree of differentiation, or the age, required of various tissues before they can respond to hormonal stimulation is receiving considerable attention in this laboratory. Other investigations in progress deal more particularly with different tissue responses to gonadal hormones, but several questions of importance relate to responsiveness of gonads to gonadotropic substances. Smith and Engle (1) demonstrated clearly that rat testes, but more especially rat ovaries, could be stimulated easily by the 21st day of post-natal life to secrete gonadal hormones; precocious maturity of germ cells occurred in the ovary. When animals were treated at earlier ages, longer periods of treatment were required for response.

In association with the problems of tissue responses to hormone during reproductive development in the opossum (2, 3), a pertinent question arose as to the period at which gonad hormones were first secreted. The suggestion was made that perhaps one would be justified in assuming that a gonad did not secrete hormone at stages when it would not react to gonadotropic substances. Gonadotropic treatments had been carried out on a few males and females at that time, but it was admitted that the materials were not wholly satisfactory, and treated animals were too few to do more than suggest that the opossum gonads failed to show responses to gonadotropic treatments prior to a post-natal age of approximately 100 days. Because of the incompleteness of this portion of the study, and to gain additional information, a series of male and female pouch-young opossums and a few older specimens have been subjected to treatments with gonadotropic agents for varying lengths of time, and dosage, during the early periods of sexual differentiation. The present account deals with a consideration of the effects indicated from such treatments.

EXPERIMENTAL METHODS AND RESULTS

Opossum pouch-young and juvenile individuals up to a post-natal age of 5 months, including both sexes, have been utilized as the experimental material. The sexually differentiating pouch young are available for direct treatment and the responsiveness of the reproductive tract during early stages in sexual differentiation to androgens and estrogens has already been proved; it is evident that when the gonads are sufficiently developed to respond to gonadotropic treatments by precocious secretion of sex hormones, it can be detected readily by responses occurring in the different parts of the reproductive tract. The gonadotropic principle in pregnant mare serum[2] has been

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[1] This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago. Invaluable aid in these studies has been contributed by Dr. Dorothy Price.
[2] The equine gonadotropins were supplied as *a*) Gonadin, by Dr. D. H. Wonder of the Cutter Laboratories, Berkeley, Cal., and *b*), Gonadogen, by Dr. G. F. Cartland of the Upjohn Co., Kalamazoo, Mich.

employed for daily subcutaneous injections. Gonad morphology, sex ducts, and accessory reproductive organs have been examined in serial section for any type of response indicating stimulation from the gonadotropic treatments.

This report embodies the principal results noted in observations made on 14 treated males and 13 treated females compared with untreated young of similar ages. In the table pertinent data are recorded as to size, age, type and duration of treatment, as well as notations on observable effect.

Responses of Males to Gonadotropic Treatment

The most satisfactory indicator of male hormone effects in the young opossum is the prostate gland, and this structure has served as the principal indicator for excessive, or precocious, testis hormone secretion in this set of experiments. It was shown earlier (3) that the prostate gave definite responses to treatments with testosterone from *day* 3 to *15* with autopsy on *day 16*, and to treatment with testosterone propionate on *days 10* to *21* with autopsy on *day 22*.

Briefly reviewed, the prostate gland in the opossum makes its first appearance as simple epithelial buds from the urogenital sinus epithelium on *days 16* to *17* and androgenic treatment is able to hasten this first appearance. By *day 40*, or slightly earlier, the solid prostate cords begin to show branching, and, as development continues, the original solid outgrowths may show 6 or more branches growing freely through the mesenchyme towards the peripheral boundary of the urogenital sinus. At the 100th day after birth (fig. 2) the tree-like branches of solid epithelial cords are well developed, but 4 males at this age failed to show hollowing out of the cords, or canalization, which begins at the central ends attached to the lining of the urogenital sinus (for canalization see fig. 1); these central openings constitute the future duct openings into the urogenital canal. By *day 125* two normal males exhibited beginnings of canalization which, in one, extended appreciably toward the peripheral branches of the original outgrowths. By the 5th month canalization was well advanced and rapid growth had caused the originally quite straight duct portions to develop decided convolutions, such that immediately outside the urogenital sinus cavity numerous cross sections of the convoluted portions were evident. The prostate of the 8-month-old opossum reveals that canalization has extended to the peripheral ends of prostate cords which are now assembled in closely packed columns; gland cells have differentiated into secretory cells quite characteristic of mature adult secreting prostates.

In the table under column headed 'response' it is revealed that the earliest unquestioned response to the equine gonadotropic treatment occurred in *animal 201A*, autopsied on *day 70*. The condition of the prostate in this animal (fig. 1) clearly demonstrates a precocious male hormone secretion. Since the prostate at this age is not

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Fig. 1. UROGENITAL SINUS of a male opossum treated with equine gonadotropin and autopsied on *day 70* (*ser. 201A*); precocious development of the prostate gland. Peripheral ends of cords arranged in columns, central ends canalized. Compare with untreated 100-day-old normal male, figure 2. ×22.

Fig. 2. UROGENITAL SINUS of a normal male opossum 100-days-old (*ser. 82A*) to show branching, uncanalized prostate cords attached to epithelium of urogenital canal. ×22.

Fig. 3. OVARY of 5-month-old opossum (*ser. 164*) after 4 daily treatments with equine gonadotropin. Follicular stimulation is marked in comparison with untreated control of same age, but luteinization is only begininng. ×18.

Fig. 4. OVARY from a 100-day-old female opossum (*ser. 90*) receiving 15 daily treatments with equine gonadotropin, without showing follicular stimulation. Except for relative absence of large cell nests this would represent a 100-day-old untreated ovary. ×18.

Fig. 5. UTERUS of a 125-day-old opossum (*ser. 231*) after 13 daily treatments with equine gonadotropin. The precocious development is evident from a comparison with the untreated control uterus, figure 6, under similar magnification. ×12.

Fig. 6. UTERUS of a 125-day-old untreated opossum (*ser. 229*). ×12.

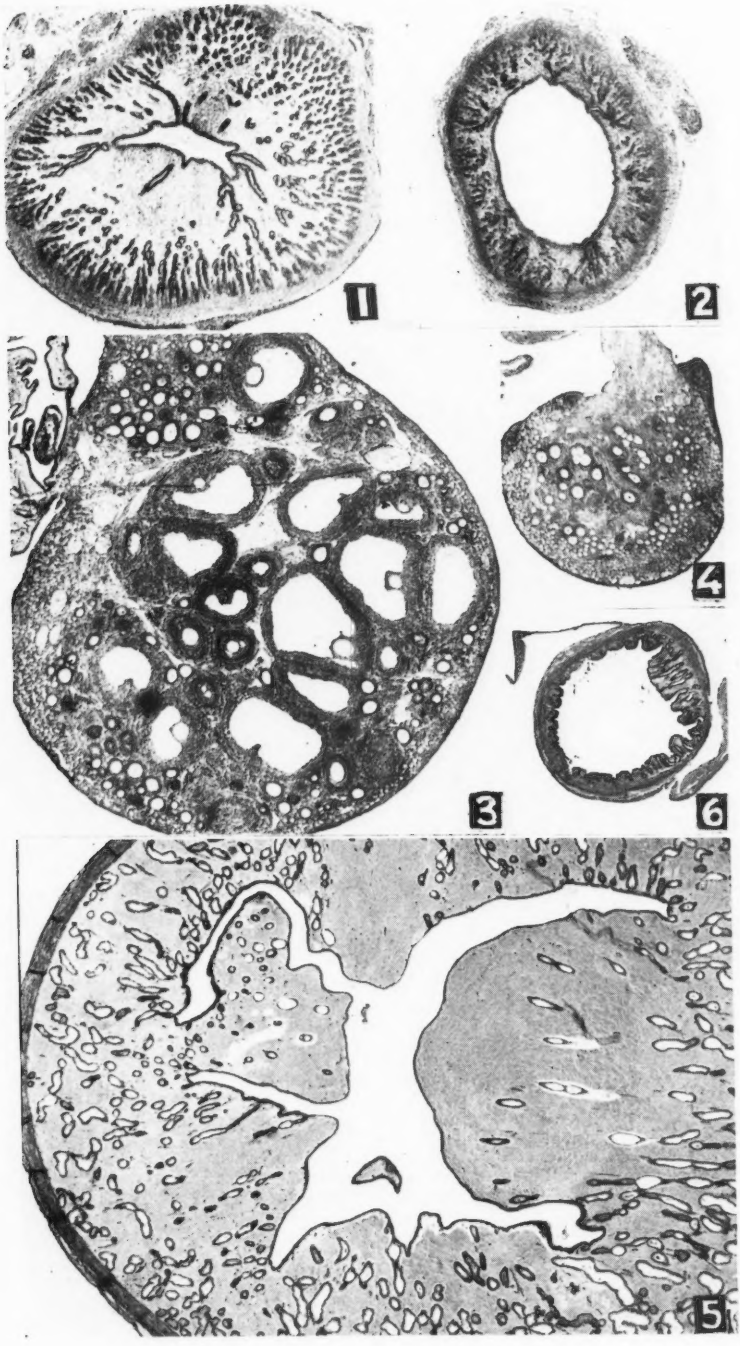
1
2
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TABLE 1. DEVELOPING OPOSSUMS TREATED WITH EQUINE GONADOTROPIN

Animal Number	Age at Autopsy, days	Snout-Rump Length, mm.	Head Length, mm.	Body Weight, gm.	Equine Gonadotropin, Days	Equine Gonadotropin, Dosage, R.U.	Weight 2 Gonads (milligrams)	Weight Reproductive Tract, Minus Gonads (mgs.)	Hormone Response
				Males					
129A	15	30.5	9.8	1.17	8–14	10[1]			0
99A	17	34.0	11.5	1.90	10–16	1[2]			0
102A	21	41.0	13.5	2.43	10–20	1[2]			0
112	30	39.5	13.5	1.97	15–29	5			0
196A	63	98.0	32.4	24.44	54–62	10			?
201A	70	115.0	36.0	34.00	54–69	10			+
202	80	148.0	47.0	71.00	54–79	10			+
203	80	148.0	46.5	82.00	54–79	10			+
204	80	144.0	45.2	68.00	54–79	10			+
89	100	175.0	52.5	140.00	85–99	5[2]			+
226	125	254.0	69.6	276.00	112–124	5	351	1430	+
227	125	226.0	69.5	300.00	112–124	5	210	1190	+
224	125	241.0	71.4	372.00	normal control		292	810	
225	125	237.0	67.5	340.00	normal control		288	600	
168	5 mo.			356.00	14 days	40	443	740[3]	+
169	5 mo.			172.00	14 days	40	496	473[3]	+
176	5 mo.			370.00	normal control		513	107[3]	
177	5 mo.			345.00	normal control		464	94[3]	
				Females					
129B	15	29.8	10.0	1.13	8–14	10			0
99B	17	37.0	11.6	2.00	10–16	1[2]			0
102B	21	41.2	13.5	2.58	10–20	1[2]			0
113	30	43.5	15.2	2.93	15–29	5			0
116	38	59.7	20.1	6.40	15–37	5			0
196B	63	95.0	32.3	23.75	54–62	10			0
201B	70	115.0	37.0	36.00	54–69	10			0
205	80	196.0	46.0	78.00	54–79	10			0
90	100	173.0	52.0	130.00	85–99	5[2]			0
230	125	225.0	68.6	220.00	112–124	5	819	4350	+
231	125	227.0	67.8	240.00	112–124	5	584	4800	+
228	125	245.0	74.7	320.00	normal control		30	470	
229	125	230.0	72.6	300.00	normal control		24	360	
164	5 mo.			240.00	4 days	40	221	2160	+
167	5 mo.			270.00	13 days	40	1040	7000	+
178	5 mo.			410.00	normal control		60	338	
179	5 mo.			390.00	normal control		76	416	

[1] Gonadin used except in cases as indicated.
[2] Gonadogen, 1 R.U. roughly equivalent to 10 R.U. of gonadin.
[3] Prostate gland only.

easily separated for weighing, some evidence for a response is based upon the finding that the number of sections in the treated prostate is 70 per cent greater than in an untreated male of the same age.

Histologically, the precocious development of this prostate was sharply indicated. A comparison of figure 1, which is a cross section through this prostate, with figure 2, a section through the prostate of a 100-day-old normal male (*ser. 82A*), reveals that peripherally the branchings of this treated gland are arranged in definite columns without peripheral canalization, but that lumina have already developed centrally in the duct portions and open widely into the cavity of the urogenital sinus; the marked difference between the treated and considerably older normal control gland will be evident. Because of similar magnification actual size differences are striking. Such a

development as revealed in this prostate of a treated 70-day-old animal compares favorably with the normal developing gland of a male 125 days or older.

The testes of this treated 70-day-old male failed to show very marked differences from normal testes of this age. Seminiferous tubules were without lumina (occur normally by the 5th month) and spermatogenesis was not advanced over that in the control; the germinal epithelium was from one to two cells in depth and the general picture of mild mitotic activity prevailed, as it did in the normal organ. Interstitial cells were apparently more abundant and were somewhat larger than in testes of normal untreated males of this age. The average diameter of 25 seminiferous tubules was slightly greater in the testes of the treated animals than in the testes of the normal control.

In brief, therefore, the equine gonadotropic treatments applied to this male, autopsied on *day* 70, induced only very slight morphological changes in the testes but caused a sufficient hormonal response to be clearly registered in the histological state of the prostate gland.

Prior to day 70. Males receiving equine gonadotropic treatments and killed before the age of 70 days, with one possible exception, failed to indicate precocious prostate development; unquestioned morphological responses failed to occur in the testes. In no case within this group was spermatogenesis advanced by treatment nor were interstitial cells unquestionably modified. The average diameter of 25 seminiferous tubules in some cases showed a slightly greater size of the tubules in treated animals than in controls of the same age (particularly in *102A*, killed on *day 21*); but in the absence of a consistent response of this nature, or of other detectable responses, no great significance was attached to these occasional differences in tubule size.

The possible exceptional case in the group of earlier aged animals noted above was *animal 196A*, killed on *day* 63 after 9 days of treatment, in which the response to equine gonadotropic treatment was rated as questionable. The prostate gland appeared in fewer serial sections than in 2 untreated males (434 sections in treated; 51[illegible] in a 67-day-old normal and 504 in a 77-day-old normal) but the outgrowths in the treated animal appeared to be somewhat further developed than in controls. Whereas the differences were not great between treated and controls, the animal in question has been rated 'suggested prostate stimulation.' Since the gland is known to respond quickly and definitely to application of androgenic substances, it is clear that very little, if any, precocious hormone secretion by the testis had actually occurred. This case was therefore doubtful in indicating a detectable response to the equine gonadotropic treatments.

Subsequent to day 70. In each of the 8 males treated with equine gonadotropic substance and killed subsequent to 70 days of age, the prostate gland was seen to be precociously developed. *Animal 204*, the smallest of 3 similarly treated males autopsied on *day* 80, had three times as many sections containing prostate tissue as did the untreated control; and in differentiation, the prostate of this 80-day-old treated male exceeded that of the 125-day-old normal control and was similar in development to the prostate of the 5-month-old normal animal.

In the two males treated for the preceding 13 days and killed on *day 125* the average weight of the reproductive tract was double that of the average of 2 normal controls. The average weight of the prostate gland of the 2 males receiving 14 daily treatments and autopsied at an age of 5 months was more than 6 times the average prostate weight of 2 untreated controls. Prostate stimulation was, therefore, evident from gross size as well as from histological development in all treated males killed at an age of 70 days or older, and the responses are to be attributed to precocious secretion of male hormone by the testis as a response to the equine gonadotropic treatments.

Testis response in these older treated males was indicated chiefly by interstitial cell hypertrophy. Spermatogenesis was not hastened in any case. The average diameter of 25 seminiferous tubules, measured with ocular micrometer, was increased slightly in some cases when compared with the controls but this was by no means consistent; increases were noted in 80-day-old and 100-day-old animals, but did not occur in the males autopsied on the 125th day or at 5 months. The total weight of the testes was not increased by the equine gonadotropic treatments in the 2 older groups in which weights were available.

Responses of Females to Equine Gonadotropic Treatment

Notations in the table reveal the fact that 9 female opossums killed on *days 15* to *100*, after receiving daily treatments with equine gonadotropin from 7 to 26 days prior to autopsy, failed to exhibit any evident differences in the histological differentiation, or in sizes of the lumina of the uterus, cervix or lateral vaginal canal. In each case ocular micrometer measurements of lumina throughout the Müllerian duct derivatives were recorded. Previous observations (2–7) had revealed that this reproductive tube during early stages of development was very sensitive to applied estrogens, as well as to applied androgens. Thus, the ovary up to an age of 100 days failed to respond to the influence of equine gonadotropin by secretion of ovarian hormones in detectable amounts.

The ovary of treated females killed from *day 63* to *100* did reveal some histological responses to these treatments, but the responses were in no demonstrable manner associated with hormone secretion; such changes were indicated, although not so definitely, in the female autopsied on *day 38*. The nature of the histological responses consisted of a more rapid breaking apart of the large-celled secondary sex cords, or cell nests, in the young ovary (for details of the normal changes in the developing ovary see reference 8). Morgan has described the development of such very prominent cell nests in the normal ovary as a gradual pinching off at the periphery of the masses of individual enlarged cells through an invasion of the edges of the masses by stromal cells that break up the nests into very small individual follicles; while this is in progress other nearby follicles are already quite definite and the single layered granulosum may have changed from the somewhat indistinct, flat-layered granulosum into a single, compact, columnar epithelium surrounding the egg cell. In these ovaries showing obvious responses to the equine gonadotropic treatment the dissolution or breaking up of the cell nests into individual primary follicles was materially advanced over that in the ovaries of controls of similar age. However, no evidence is at hand to indicate that the stratum granulosum was stimulated to an enhanced rate of cell division. The normal ovary at 100 days of age (see fig. 4) in several females failed to approach the stage of development in which antra were present in the follicles; in fact, seldom at this age was a follicle seen that had a stratum granulosum of even two cells in thickness. Antrum formation in normal ovaries was present at *day 125*, hence the attainment of this stage of follicular development occurs between the 100th and the 125th day of development.

Thus, although histological changes were evident in the ovaries of females treated with equine gonadotropin prior to autopsy on *days 63* to *100*, the response was limited to a precocious breaking up of cell nests into individual primordial follicles; it did not involve any apparent stimulation of granulosa cells or earlier appearance of antra in the follicles, and there were no detectable evidences for secretion of ovarian hormones.

Day 125. Equine gonadotropin administered to 2 females daily for 13 days prior to autopsy on *day 125* induced striking changes in the reproductive tract, both in respect to ovarian size and to marked advance in histological differentiation in the

reproductive tract. Precocious ovarian hormone secretion is apparent, therefore, at *day 125* and represents the earliest detectable evidence for secretory stimulation of hormones by the ovary as a response to equine gonadotropins.

At this age the entire female reproductive tract was relatively easily dissected from the animal, and individual parts could be weighed on a torsion balance. Attention to the table will reveal that the average weight of the ovaries in 2 females treated with equine gonadotropin, in comparison with the average ovarian weight in 2 normale females of the same group of animals, was 26 times greater (average wt. of normal ovaries 27 mg.; treated, 701 mg.); the average weight of the reproductive tract (without ovaries) showed that of the treated females to be 11 times greater than that of the controls (average for normal tract, 415 mg.; treated, 4575 mg.).

A study of ovarian histology in the normal 125-day-old female reveals that antrum-bearing follicles are fairly numerous and that many of them show the usual prepuberal atresia, but there were no evidences for luteinization. In the treated animal of the same age one finds in the ovary inactive primordial follicles; follicles with single-layered, columnar granulosum; a granulosum of many cells in thickness; follicles with normal-appearing antra; antrum-bearing follicles with beginning luteinization becoming evident; follicles with advanced luteinization, but showing connective tissue-like fibrillar centers; and solid corpora lutea containing the inclosed egg cell. At this stage, therefore, the ovary proves to be decidedly responsive to the equine gonadotropic treatments.

The reproductive tract of the normal 125-day-old females revealed a uterus with small, although definite, uterine glands (fig. 6); these have not been seen in normal females at 100 days of age, nor in 100-day-old females treated with equine gonadotropin. We have not yet determined whether uterine gland formation requires the stimulating action of estrogenic hormones. The epithelium of the lateral vaginal canals of normal 125-day-old females was relatively simple and apparently inactive; the surrounding connective tissue was moderately compact or condensed. In the treated females the uterus (fig. 5) was much larger and the glands had increased enormously in size, having individual cells that had the definite morphology of the secretory gland. In lateral vaginal canals the epithelium had increased to from 3 to 6 cell-layers and marked cornification and sloughing off of cells into the lumen had occurred; the surrounding connective tissue was less compact and, as in the uterus, it was edematous.

It thus becomes evident that the 125-day-old female exhibits an enormous response on the part of the reproductive system to treatments with equine gonadotropin; the ovary has been stimulated in follicular development and in luteinization, and the ovarian hormones secreted under the stimulus of equine gonadotropins have exerted a marked influence on the reproductive tract. This age represents the earliest one in which definite evidence for ovarian stimulation in these respects has been obtained; in this case, there is no question relative to the capacity of the ovary to respond to equine gonadotropins, for the evidence of precocious secretion is incontrovertible.

Five months of age. Two females slightly over 5 months of age were subjected to treatment; one received treatment for only 4 days and the second for 13 days, with a greater dosage of equine gonadotropin than in other cases. Two untreated females from the same group of animals served as controls and were autopsied at the time of killing the longer-treated female.

The data recorded in the table indicate that ovarian weight in the female subjected to 4 days of treatment was more than 3 times the weight of ovaries from the untreated controls, whereas the weight of ovaries from the female receiving treatment for 13 days was 15 times the average weight of ovaries from the 2 control females. The

weight of the reproductive tract (without ovaries) of the female treated for a short time was greater than 5 times the control average, and the reproductive tract from the female treated for the longer period was almost 20 times the average weight of the control reproductive tracts.

Histological examination of the ovary (fig. 3) of the animal subjected to equine gonadotropin treatment for only 4 days revealed a very definite increase in number of antrum-bearing follicles, and follicular size was also greater. No apparently imminent ovulation was detected since, for the most part, the enlarged follicles were situated relatively deeply in the ovary. Luteinization was only suggested in a few of the larger follicles and no definite well-formed corpora lutea were seen. The ovary subjected to the influence of equine gonadotropins for a period of 13 days, was characterized by almost solid luteinization. A few relatively large follicles exhibited beginning luteinization, but for the most part unluteinized follicles were small, and, in the majority of cases, were devoid of antra.

DISCUSSION

One of the interests foremost in mind in undertaking this study was the desire to gain some information relative to the age of the animal, and the state of the gonad, when first the gonads begin to secrete sex hormones. The question whether the gonads secrete hormones during embryonic development has never been settled and the problem presents many difficulties. Definite criteria for the beginning of hormone secretion are difficult to establish. It has been assumed generally that gonads during embryonic development secrete their sex-specific hormones responsible for directing the development of the two opposite sets of embryonic sex-ducts, such that along with differentiating ovaries the Müllerian ducts prosper and the Wolffian ducts are extinguished; or, that, in the development of the testes, hormones secreted by them promote the growth of the male system and exclude through degeneration the female, or Müllerian ducts. This assumption is curiously devoid of proof and Moore (3) has presented evidence favoring the interpretation that early duct, or accessory reproductive organ, development is not controlled by gonadal-secreted hormones.

In the rat, one of the more frequently studied animals, it is still uncertain whether the gonads at birth are actively secreting sex hormones and, to the knowledge of the writers, this has not been satisfactorily determined for any mammal, or for any bird at the time of hatching; what attention these problems have received has usually involved post-natal or post-hatching stages. Domm and Dennis (9) injected pituitary hebin into developing chicken eggs and noted that whereas both testes and ovaries in the chick at the 16th day of incubation were frequently increased in size as a response to gonadotropic substances, the duct system and comb size failed to indicate any response that could be attributed to hormones secreted by the gonads; sex hormones introduced into developing eggs induced comb-growth and increased duct-size, hence it was apparent that gonadal secretion had not been stimulated by the pre-hatching treatment with gonadotropic substances.

The opossum, because of its extreme immaturity in sexual differentiation at birth, provides excellent materials upon which to conduct such studies. It should be recalled (3) that *a*), at birth the gonads are not clearly distinguishable as ovary or testis in histological preparations; *b*), that Müllerian ducts show their first appearance anteriorly in both sexes about *day* 3 and reach the urogenital sinus connections about *day* 10; and *c*), that Wolffian ducts in both sexes function as urinary ducts for approximately 2 weeks after birth. Since these tiny new-born young are available for treatment at such early stages in sexual differentiation they present exceptional possibilities for determining the onset of active secretion by the developing gonads.

When one attempts to select a definite criterion for the earliest secretion of hormones by the gonad, it is natural to expect it among the structures of the reproductive

system, the control of which during maturity is largely dependent on gonad hormone secretions. But what criterion is dependable? Moore (10) has already shown that in males castrated at the time of appearance of the prostate as small buds from the urogenital sinus epithelium (*day* 20) this gland continues to grow and differentiate in a manner similar to that in the normal male, at least up to 50 days of age. Unpublished observations will extend this to considerably later periods and will also reveal that differentiation of the female reproductive tract proceeds uninterruptedly after ovarian removals on *days* 20 to 22.

At the present time, information in hand strongly suggests a lack of any evidence for gonad hormone secretion in the untreated opossum prior to *day* 100; what individual, or set of, criteria may ultimately prove the most dependable is not now known.

The suggestion was made earlier (3) that gonads which were incapable of precocious hormone secretion as a result of gonadotropic stimulation probably were not secreting sex hormones. Although the suggestion has not been proved, all the evidence known to us favors the assumption. Thus, gonadotropic treatments of newly hatched chicks stimulated testes to secrete hormones and the androgens thereby secreted were sufficient to produce enlarged combs and wattles within 10 days of age and to induce attempts on the part of the treated young males to crow and tread their mates (11, 12); young male rats treated with gonadotropic substances showed sufficient responses on testis hormone secretion to bring forth secretion granules in seminal vesicles by the 12th post-natal day, whereas these appear normally about *day* 35 to 40 (13); adult male rats not secreting hormone because of nutritional difficulties (vitamine B-free diets or inanition) responded at once with gonad secretions if treated with gonadotropic substances (14, 15); ground squirrels, immature or in seasonal quiescence, showed testis hormone secretions within a few days after receiving gonadotropic treatments (16, 17). In female rats the gonads were even more rapidly stimulated by gonadotropic substance to secrete sex hormones. By the 21st day ovarian hormones were readily apparent after short treatments with gonadotropins but in rats implanted with pituitary tissue at earlier ages a longer period was required to gain similar responses (1). The numerous reports of the effectiveness in awakening gonadal secretion in both hypophysectomized male and female animals, and the lack of any known cases in which gonads failed to respond to gonadotropins, irrespective of whether they have previously secreted hormones, emphasize the notion that gonads capable of secreting hormones will respond to gonadotropic stimulation; we are unaware of experiments other than those of Domm and Dennis (9) on the embryonic chick in which gonadotropic treatment has been carried out at sufficiently early stages in development that it would fall in the period prior to that in which the gonads could respond.

In an attempt, therefore, to determine at what period in development the opossum gonad is capable of secreting hormones as a result of gonadotropic stimulation we have relied upon a comparison of the accessory organs of reproduction, which have proved capacity to respond readily to small quantities of pure chemical androgens and estrogens. A positive response in gonadotropic-treated young would involve a response on the part of the accessory reproductive structures similar to those induced by direct treatments with androgens or estrogens, and would represent precocious development in comparison with normal controls of similar age.

In young, male opossums treated with equine gonadotropins, unquestioned androgenic secretion has been elicited as early as *day* 70 and some evidence was suggested by *day* 63; in young treated females unquestioned precocious secretion of ovarian hormones has not been noted until subsequent to *day* 100. By *day* 125 pronounced effects from the treatments demonstrated the awakening of ovarian secretions.

The evidence available at present suggests that the gonads prior to these periods (testes, *day 63*; ovaries, *day 100–125*) are incapable of hormonal response to equine gonadotropic treatments, and in consequence it would also appear to indicate strongly that the gonads under normal conditions are incapable of secreting hormones prior to this period. Since the reproductive tract of both males and females is well along in development at this age, further evidence is provided for the notion that sex-duct and accessory-reproductive-organ development at early stages in sexual differentiation are not under the control of gonadal secreted hormones.

SUMMARY AND CONCLUSIONS

Fourteen male and 13 female pouch-young and juvenile opossums have been treated with equine gonadotropin for varying periods between the 8th and 150th day of life.

The condition of the prostate gland in the male demonstrates that testes can respond to equine gonadotropins by precocious secretion of male sex hormones as early as *day 70*; slight indications suggested a mild response as early as *day 63*. The condition of the reproductive tract in females proved that the ovaries could respond to equinine gonadotropic stimulation by precocious sex-hormone secretion subsequent to *day 100*. Previous to *day 100* the treatments caused precocious conversion of embryonic egg-nests into primordial follicles, but did not stimulate hormone secretion in detectable amounts.

Since it appears justified to believe that gonads incapable of secreting hormones precociously as a result of equine gonadotropic stimulation do not normally secrete hormones at such periods, it would follow that the early differentiation of sex ducts and accessory reproductive organs in the opossum could not be controlled by gonadal secreted hormones.

REFERENCES

1. Smith, P. E., and E. T. Engle: *Am. J. Anat.* 40: 159. 1927.
2. Moore, C. R.: *Proc. Soc. Exper. Biol. & Med.* 40: 544. 1939.
3. Moore, C. R.: *Physiol. Zoöl.* 14: 1. 1941.
4. Burns, R. K., Jr.: *Proc. Soc. Exper. Biol. & Med.* 41: 60. 1939.
5. Burns, R. K., Jr.: *Proc. Soc. Exper. Biol. & Med.* 41: 270. 1939.
6. Burns, R. K., Jr.: *J. Morphol.* 65: 79. 1939.
7. Burns, R. K., Jr.: *J. Morphol.* 65: 497. 1939.
8. Morgan, Chas. F.: In press.
9. Domm, L. V., and E. A. Dennis: *Proc. Soc. Exper. Biol. & Med.* 36: 766. 1937.
10. Moore, C. R.: *Anat. Rec.* 80: 315. 1941.
11. Domm, L. V., and H. B. Van Dyke: *Proc. Soc. Exper. Biol. & Med.* 30: 349. 1932.
12. Domm, L. V.: *Cold Spring Harbor Symposium on Quant. Biol.* 5: 241. 1937.
13. Price, Dorothy: *Am. J. Anat.* 60: 79. 1936.
14. Marrian, G. F., and A. S. Parkes: *Proc. Roy. Soc., London, s. B.* 105: 248. 1929.
15. Moore, C. R., and L. T. Samuels: *Am. J. Physiol.* 96: 278. 1931.
16. Wells, L. J.: *Anat. Rec.* 62: 409. 1935.
17. Wells, L. J., and C. R. Moore: *Anat. Rec.* 66: 181. 1936.

EFFECT OF OPTICAL STIMULI ON OUTPUT OF URINE IN ALBINO RATS

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Boyd and Young (1) have shown that optical stimuli in the form of repeated flashes of bright light affect the water balance of frogs. They presented evidence which suggested that the reaction was brought about through an optico-hypothalamico-hypophysial nervous pathway liberating water-balance factors from the neurohypophysis. In the present communication data will be presented upon the effect of corresponding optical stimuli upon the rate of urine formation in albino rats.

Healthy albino rats weighing about 200 gm. were used. They were assembled in a dark room beneath an inverted cone 18 inches high which contained at its apex a 200-watt bulb connected in series with a Brodie cut-out key which revolved at a rate sufficient to give a brief flash of light every 5 seconds. Sufficient draft ventilation was present to prevent any change in the temperature of the air over the animals, this being constantly checked by a thermometer attached to the cone. The animals were placed under the cone in a large funnel which had two wire floors, one of large mesh about ⅔ of the way down the funnel upon which the rats were placed and a fine wire mesh floor at the bottom of the funnel which trapped feces but allowed urine to flow through and be collected in a graduated cylinder beneath the funnel.

When albino rats were arranged as above and subjected to flashing of the light in the daytime, most of the animals were found asleep in a few minutes—at least they were lying still with their eyes shut. The flashing produced no obvious excitement and had no effect upon the rate of urine formation of such animals. To keep the animals awake, or to keep their eyes open, three procedures were tried. *a*), Experiments were performed during the night when rats are normally awake; the method proved satisfactory for short, but not for long-continued, use. *b*) Analeptic drugs were given in an effort to awaken the animals during the daytime. Most of the analeptics used, including caffeine, nikethamide and leptazol, had a diuretic effect which was undesirable in experiments designed to measure urine volume. *c*) The eyelids were removed from rats so that, when the animals were used several days later, they could not prevent light from reaching their eyes. This method proved most satisfactory. Some of the rats in time developed infection of the eyes and were discarded. Many survived for weeks, new eyelids gradually growing which had to be removed again. To prevent the animls from turning their heads away from the light, they were placed in wire strait-jackets which were just large enough to hold a rat, but not large enough for the animal to turn or move about. The rats in strait-jackets were placed in the funnel with their faces directed upward toward the light. For each rat subjected to flashing, another rat, similarly treated in all particulars except for exposure to flashing light, was used as a control.

Experiments were initially performed upon rats which had been fasted and dehy-

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drated overnight, but the rate of urine formation was so small in these animals that in all subsequent experiments the animals were deprived of food but not of water overnight before use. To overcome variation in the amount of water which the rats might have taken by chance just before being used, large numbers of animals were employed in the control and flashed groups. Most of the animals emptied their bladders during the excitement of being arranged in the apparatus so that there was generally little output of urine during the first hour or two of the experiment.

The results obtained with 214 rats treated as described above, half of which were exposed to flashing light and half controls, have been summarized in table 1. During

TABLE 1. EFFECT OF OPTICAL STIMULI UPON THE MEAN HOURLY RATE OF OUTPUT OF URINE IN ALBINO RATS

Hour of Flashing	1st	2nd	3rd	4th	5th	6th
Rats exposed to flashing light, cc./kg./hr.	0.37	1.37	1.31	0.70	1.1	1.4
Unexposed rats, cc./kg./hr.	0.22	0.68	1.08	1.4	1.4	1.6
Mean difference, cc./kg./hr.	+0.15	+0.69	+0.23	−0.70	−0.3	−0.2
Mean difference, % of control by exposed	+68	+101	+21	−50	−21	−12

the first hour of flashing of the light, the rats excreted a mean of 0.37 cc. of urine per kg. of body weight, the unexposed controls, a mean of 0.22 cc. per kg. The mean difference of 0.15 cc. was 68 per cent of the control value or, in other words, the rats exposed to a flashing light excreted a mean of 68 per cent more urine than did the unexposed controls during the first hour. During the second hour, the difference was more striking, the rats exposed to the flashing light producing 101 per cent more urine than the control animals. Flashing light during the third hour had less effect. In the fourth hour, the rats exposed to flashing light excreted only half as much urine as the controls; during the 5th hour 78 per cent, and the 6th hour 87 per cent of the output of the controls.

The results indicate that over a period of 3 hours, flashing light increased the output of urine, during the next 3 hours the rate decreased so that at the end of 6 hours the total urine output of rats exposed to flashing light and unexposed rats was about the same, approximately a mean of 6 cc. per kg. of body weight. It was possible to show that several of the mean differences, which were sufficiently great, were statistically significant. Thus, during the second hour, the mean difference between the urine volume of experimental and control rats was 0.69 cc. per kg. and it had a standard deviation of 0.29, which is indicative of a significant difference, P being less than 0.05.

The most likely interpretation of these results is that they were due to a neural reflex originating in the eyes. The possible pathways of reflexes originating in the eyes are many. The two primary centers are the lateral geniculate bodies and the superior colliculi. From the lateral geniculate bodies, impulses may pass to the supraoptic nucleus of the hypothalamus via the dorsal supraoptic commissure (2). From the superior colliculi, impulses may pass along the tectospinal tract, the medial longitudinal fasciculus and the tectocerebellar tracts. The tectospinal tract makes various connections possible, for example from the red nucleus to the tuberal nuclei of the hypothalamus (2). Through the medial longitudinal fasciculus, many other pathways are possible, including those to the thoracolumbar sympathetic outflow via the lateral ground bundle.

Of the various possible pathways, only one has been investigated, the pathway

or pathways to the hypothalamic nuclei and thence by the median eminence and infundibular stalk to the neurohypophysis. Impulses passing along this possible pathway might conceivably cause the neurohypophysis to secrete the hormone affecting water balance.

Although it seemed obvious enough that the effect of flashing light was mediated through optical stimuli, to make certain that this was the case the eyes were removed from a group of 96 rats; half of these animals were exposed to flashing light and half retained as unexposed controls. In this experiment, the flashing of light had no effect upon the rate of urine formation.

Heller (3) has shown that the rat hypophysis contains the equivalent of about 0.5 international units (I.U.) of posterior pituitary extract per 100 grams of body weight of rat. On the hypothesis that optical stimuli might have caused release of this, or neighboring amounts of the posterior pituitary water balance principle, pituitrin,[1] was injected into unflashed rats assembled as above and in doses of from 10^{-18} to 1

TABLE 2. EFFECT OF VARIOUS DOSAGE LEVELS OF POSTERIOR PITUITARY EXTRACT UPON THE MEAN RATE OF URINE FORMATION IN THE NORMAL ALBINO RAT

Dosage, I.U./100 gm. Body Weight	Rate of Urine Formation, cc./kg./hr.	
	Average over 1st, 2nd and 3rd hr.	Average over 4th, 5th and 6th hr.
0	0.8	0.6
10^{-18} to 10^{-15}	0.6	0.8
10^{-14} to 10^{-12}	0.6	1.0
10^{-11} to 10^{-9}	0.4	0.7
10^{-8} to 10^{-6}	0.9	1.3
10^{-5} to 10^{-3}	0.8	1.2
10^{-2}	1.8	1.3
10^{-1}	2.5	1.7
1	4.3	0.5

I.U. per 100 gm. of body weight, in a total of 344 rats. It is well known that pituitrin inhibits water diuresis in rats, and it is commonly held to have no effect upon the rate of urine formation in normal animals. Doses of pituitrin up to and including 0.001 I.U. per 100 gm. of body weight had no consistent effect upon the rate of urine formation. Doses of 0.01, 0.1 and 1 I.U. per 100 grams of rat produced an immediate diuresis in normal rats arranged as for the flashing of light but the light was not flashed. The effect of these various doses of pituitrin is summarized in table 2. Control animals were given an equivalent volume of pituitrin vehicle which slightly increased the normal output of urine in the first few hours. These results indicate that amounts posterior pituitary extract of the same order as those present in the rat hypophysis can produce a diuresis in rats, just as did the flashing of light into the eyes. It is possible, therefore, that the diuresis from optical stimulation may have been due to the release of water balance principle from the rat's own hypophysis.

If this were so, then the effect of flashing light might correspond to the effect of injecting an extract of the total posterior pituitary gland of the rat. To investigate this, the posterior pituitary glands of rats were extracted with pituitrin vehicle and injected into other rats arranged for exposure to the flashing light but the light was not flashed. The total extract from one rat was injected into another rat and the rate of urine formation noted, controls consisting of rats injected with pituitrin vehicle. The results have been summarized in table 3 in which it may be seen that an extract of the whole posterior pituitary gland of the rat caused diuresis in normal rats. Thus,

[1] Pituitrin, N.N.R.

TABLE 3. EFFECT UPON THE MEAN RATE OF URINARY OUTPUT IN ALBINO RATS OF EXTRACTS OF THE POSTERIOR PITUITARY GLAND OF ALBINO RATS WHOSE EYES HAD BEEN PREVIOUSLY EXPOSED TO A FLASHING LIGHT

Pituitary Extract Injected	Rate of Urinary Output, cc./kg./hr.		
	1st and 2nd hr.	3rd and 4th hr.	5th and 6th hr.
Pituitrin vehicle	0.6	0.5	0.7
From unexposed rats	1.8	0.9	1.2
From rats exposed for 1 hour	1.1	2.0	1.4
From rats exposed for 2 hours	3.0	0.1	3.0
From rats exposed for 3 hours	2.6	1.0	2.1

during the first and second hours, the control rats excreted a mean of 0.6 cc. of urine per kg. of body weight per hour, while those rats injected with the extract of a whole posterior pituitary gland of the rat excreted a mean of 1.8 cc. of urine per kg. of body weight per hour.

If optical stimuli reflexly caused secretion of water balance principle from the posterior pituitary gland of rats, then it might be possible to show that after optical stimulation, the pituitary gland contained an altered amount of water balance principle. To investigate this, rats were exposed to the flashing light as described above,

TABLE 4. EFFECT OF OPTICAL STIMULI UPON THE HOURLY OUTPUT OF URINE (CC./KG.) OF ALBINO RATS GIVEN WATER BY MOUTH OR KEPT AT 4°C.

Hour of Flashing	1st	2nd	3rd	4th	5th	6th
Rats receiving water by mouth and flashing light	17.8	7.3	5.1	3.5	2.0	1.9
Rats receiving water by mouth and no flashing light	18.5	8.4	4.1	4.0	3.0	2.3
Rats exposed to 4°C. and flashing light	0.5	1.6	3.2	1.3	2.4	1.9
Rats exposed to 4°C. but not to flashing light	0.5	2.9	2.3	1.9	1.6	2.3

and after 1, 2 and 3 hours the animals were killed in groups of 12 each and the posterior pituitary glands extracted with pituitrin vehicle. The extract from one rat was injected into another rat arranged for exposure to the flashing light but the light was not flashed. The mean results have been summarized in table 3. Pituitary extracts from unexposed rats caused normal rats to excrete urine at the rate of 1.8 cc. per kg. per hour during the first 2 hours after injection; pituitary extracts from rats exposed to flashing light for 1 hour caused excretion at the rate of 1.1 cc.; pituitary extracts from rats exposed to flashing light for 2 hours, caused urine excretion at the rate of 3.0 cc. and those from rats exposed to flashing light for 3 hours, at the rate of 2.6 cc. per kg. of body weight. The initial effect of flashing light seemed to be to decrease somewhat the pituitary content of the diuretic principle, while after an hour's flashing there seemed to be more of the diuretic principle in the rat posterior pituitary gland. These results indicate that optical stimulation can alter the amount of water balance principle in the neurohypophysis of the rat.

Another series of experiments was made in which the water equilibrium of rats was altered by giving them initially 5 per cent of their body weight of distilled water by stomach tube. Eighty animals were so treated, half of which were exposed to flashing light, and half were unexposed. The data for the mean hourly output of urine in these two groups have been summarized in table 4. It may be seen that optical stimulation had no effect upon the rate of urine formation in these rats given water by mouth. It seemed possible that the explanation of these results was that the release

of diuretic activity from the neurohypophysis by optical stimulation in rats could have little effect if the rats were already diuretic due to the water previously given by mouth. To investigate this suggestion, another group of 80 rats was made diuretic by placing them in a refrigerated room at a temperature of 4°C. Half of the animals were exposed to flashing light at this temperature and half left as unexposed controls. The effect upon the rate of urine formation has been summarized in table 4. Placing the rats in the refrigerated room caused a mild diuresis and flashing of light into the eyes had no effect upon the rate of urine formation.

In the explanation offered for the results described in the preceding paragraph, it has been assumed that the effect of optical stimulation would have been to cause release of a principle from the neurohypophysis with diuretic activity, but such activity was obscured by an already existing diuresis. On the other hand, it is generally held

TABLE 5. EFFECT OF PITUITRIN, N.N.R., ON THE MEAN HOURLY RATE OF OUTPUT OF URINE IN ALBINO RATS GIVEN 5 PER CENT OF THEIR BODY WEIGHT OF WATER BY MOUTH. RESULTS ARE EXPRESSED AS CC. OF URINE PER KG. OF BODY WEIGHT PER HOUR

Dose of Pituitrin I.U./100 gm. of Body Weight	Hours after Administration			
	1st	2nd	3rd	4th
0	4.0	7.4	2.3	0.4
10^{-4}	1.3	12.2	5.9	1.2
10^{-3}	0.0	16.5	4.3	1.1
10^{-2}	0.4	5.2	14.7	2.9
10^{-1}	0.8	0.0	4.6	5.8
1	6.3	4.5	0.0	1.4

that pituitrin is antidiuretic in rats which are in a state of diuresis as described. There is one qualification which must be applied to such a statement, a qualification which is not generally appreciated. It is that pituitrin is antidiuretic only over a circumscribed range of dosage. Nelson and Woods (4) noted this and Boyd, Garand and Livesey (5) found that pituitrin may increase, decrease or have no effect upon total water loss of albino rats given water by mouth depending upon the dosage of pituitrin employed. Nelson and Woods employed mice in their studies and Boyd, Garand and Livesey measured changes in terms of total body weight rather than the volume of urine. In order to have comparable data, it was necessary to investigate the effect of a range of doses of pituitrin upon the rate of urine formation in albino rats arranged for exposure to flashing light and given 5 per cent of their body weight of distilled water by stomach tube. This was done over a range of doses comparable to those found effective in table 2; the results are recorded in table 5. The experiments were performed in a warmer season of the year than those summarized in table 4 which probably accounted for the less degree of diuresis. Doses of pituitrin up to and including 0.1 I.U. per 100 gm. of body weight were found to be antidiuretic over a period of an hour or so, which is familiar to all investigators as the antidiuretic reaction. But a dose of 1 I.U. per 100 gm. was not antidiuretic, and, if anything, its action was a bit diuretic in the first hour. This is approximately the dosage found by Nelson and Woods (4) and by Boyd, Garand and Livesey (5) to have decreased antidiuretic activity. It is also in the neighborhood of the total amount of posterior pituitary water balance activity in the rat hypophysis (3). If the exposure of rats to flashing light caused secretion of amounts of water balance principle from their hypophyses corresponding to 1 I.U. of pituitrin or thereabouts, then little or no effect would be noted on the rate of urine formation if they were in a state of diuresis from the administration of water, which is precisely the result reported in table 4.

SUMMARY

Repeated optical stimuli in the form of brief flashes of bright light at brief intervals caused a diuresis over a period of 3 hours in albino rats.

This reaction did not occur if the eyes were removed from the rats.

Doses of posterior pituitary extract corresponding to the amount present in the rat hypophysis also caused a similar diuresis.

The injection of an extract from a whole pituitary gland of one rat into a rat caused a diuresis.

Optical stimuli were found to alter the amount of water balance principle which could be extracted from the rat hypophysis.

Optical stimuli had no effect upon the output of urine in rats given water by stomach tube.

Optical stimuli had no effect upon the rate of urine formation in rats which were in a state of mild diuresis from exposure to cold.

Amounts of posterior pituitary extract of the order of those found in the rat hypophysis also had no effect upon the rate of urine formation in rats given water by mouth, although smaller doses were antidiuretic in effect.

CONCLUSION

It is suggested that these results may be explained by postulating that stimuli to the eyes set up an impulse over an optico-hypothalamico-hypophysial pathway which causes secretion of water balance factors from the neurohypophysis.

REFERENCES

1. Boyd, E. M., and F. M. Young: *Endocrinology* 27: 137. 1940.
2. The Hypothalamus, *Assoc. Res. Nerv. & Ment. Dis.* Williams and Wilkins, Baltimore. 1940.
3. Heller, H.: *J. Physiol.* 100: 135. 1941.
4. Nelson, E. E., and G. G. Woods: *J. Pharmacol. & Exper. Therap.* 50: 241. 1934.
5. Boyd, E. M., N. D. Garand and R. J. Livesey: *Quart. J. Pharm. & Pharmacol.* 12: 19. 1939.

QUALITATIVE CHANGES INDUCED IN GONADOTROPIC COMPLEX OF PITUITARY BY TESTOSTERONE PROPIONATE[1]

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THE SPECIFIC effects induced in the ovaries and testes of rats by the follicle-stimulating (FSH) and luteinizing (LH) (ICSH) factors of the anterior pituitary have been repeatedly demonstrated. The reciprocal influence of the gonads on the production and liberation of these pituitary gonadotropic factors is less clear.

It is well established that the gonadotropic activity of the pituitary of the rat is increased several-fold following surgical removal of the gonads. It is equally certain that the total gonadotropic content of the pituitary gland can be decreased by giving injections of the sex hormones (1, 2). However, these investigations do not indicate how the FSH or LH are affected independently, nor do they distinguish between storage and release of these substances from the pituitary. Estrogen has been found to augment the effects of injected FSH in immature female rats as shown by increased ovarian weights and presence of luteinization (3). Also, increased formation of lutein tissue has been reported following the injection of testosterone (4, 5, and others). The evidence from the above studies indicates, especially with regard to the LH factor, that the effects of the gonads on the anterior pituitary are not limited to 'suppression' or 'inhibition.'

The present study attempts to show the influence of the testes on the pituitary by comparing the differences in the amounts of follicle-stimulating (FSH) and luteinizing (LH) factors present in the pituitaries of *a*), normal adult male rats, *b*), adult rats, castrated 2 to 5 months previously, and *c*), castrated rats treated with testosterone propionate. In addition, differences in the concentration of these gonadotropic factors in the blood serum of the above animals are reported.

EXPERIMENTAL PROCEDURE AND RESULTS

All of the donor rats were of the Sprague-Dawley strain and consisted of 71 normal males, 42 untreated castrated males and 53 males which had been castrated and injected with testosterone propionate.[2] At the termination of the injection periods, the treated animals, together with the normals and untreated castrated rats were autopsied and the pituitary glands removed. The glands were immediately

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placed in acetone, dried and powdered in a manner similar to that previously reported (6). The pituitaries were tested individually in normal and hypophysectomized immature female rats; a total of 172 normals, 21 days of age, and 54 hypophysectomized recipients were used. The hypophyses were removed when the animals were 21 days of age, and injections were started 2 days later. Aqueous suspensions of the powder were administered subcutaneously to the recipients twice daily for 3 days, and autopsies performed 2 days after the last injection. The ovaries were weighed and examined by transillumination, and those ovaries in which the presence of lutein tissue was questionable were sectioned and examined histologically.

TABLE 1. QUALITATIVE DIFFERENCES IN OVARIAN RESPONSES PRODUCED IN NORMAL 21-DAY-OLD RATS BY PITUITARIES OF *a*) NORMAL ADULT MALE RATS, *b*) CASTRATED MALES AND *c*) CASTRATED MALES TREATED WITH TESTOSTERONE PROPIONATE

Donors		Recipients						
			Number of rats showing:					
			Follicles only			Corpora lutea		
Type	Number	Average total dose pit. powder per rat	No.	Ovarian weights		No.	Ovarian weights	
				Range	Average		Range	Average
		mg.		*mg.*	*mg.*		*mg.*	*mg.*
Normal	28	3.6	12	32–46	39.5	2	64– 71	67.5
Castrated (2–5 mo.)	9	3.9	0			9	58–123	95.8
Untreated	10	1.7	0			10	39– 96	64.7
		0.9	0			12	27– 51	34.5
		0.65	0			9	18– 50	30.4
		0.34	0			10	16– 28	19.4
Castrated (2–5 mo.)	7	3.3	0			7	54–132	99.1
0.5 mg. T.P.;[1] 15 days	5	1.6	0			10	42–104	66.4
Castrated (2–5 mo.)	9	1.4	14	32–72	47.0	4	73– 89	81.8
0.5 mg. T.P.;[1] 30 days								
Castrated (2–5 mo.)	4	3.1	3	31–87	63.7	1		82.0
0.5 mg. T.P.;[1] 45 days	4	1.6	7	19–41	32.4	1		67.0
Castrated (5 days)	12	4.2	5	24–67	36.6	1		114.0
0.3 mg. T.P.;[1] 30 days								

[1] Testosterone propionate.

Gonadotropic factors in the pituitary of the normal adult male rat. The data from this group confirmed an earlier report (7) that the pituitaries of noncastrated male rats stimulate primarily follicular development in the ovaries of normal and hypophysectomized recipients. In table 1 it may be seen that the ovaries of 12 of the 14 normal 21-day-old recipients contained no corpora lutea after being injected with the pituitaries of 28 normal adult males. The amount of pituitary powder which each recipient received is shown in tables 1 and 2, and the ovarian weights are given as the average (with range) for each dose level. Twelve hypophysectomized recipients (table 2) received the pituitaries of other normal adult males, and lutein tissue was found in the ovaries of but one of these test animals. In order to produce ovarian enlargement in the hypophysectomized animals comparable, in terms of percentage increase, to that obtained in normal recipients larger doses of pituitary powder were required.

Alteration of gonadotropic hormone of the pituitaries of adult rats after castration. In contrast to the pituitaries of intact male animals, those of castrated males showed a marked increase in the luteinizing factor. In this series, the pituitaries of 19 adult males, castrated 2 to 5 months previously, were assayed in 49 normal immature females (table 1), while the pituitaries of 23 similar donors were administered to 18 hypophysectomized recipients (table 2). In order to obtain different degrees of ovarian stimulation, varying amounts of pituitary powder were injected. In the normal recipients, the doses ranged from a minimum of one-twelfth of a pituitary to a maximum of one gland, while in the hypophysectomized test animals, doses varying from one-fourth to 2 glands were injected. The resultant ovarian weights

TABLE 2. QUALITATIVE DIFFERENCES IN OVARIAN RESPONSES PRODUCED IN HYPOPHYSECTOMIZED IMMATURE RATS BY PITUITARIES OF *a*) NORMAL ADULT MALE RATS, *b*) CASTRATED MALES AND *c*) CASTRATED MALES TREATED WITH TESTOSTERONE PROPIONATE

Donors		Recipients						
			Number of rats showing:					
			Follicles only			Corpora lutea		
Type	Number	Avg. total dose pit. powder per rat	No.	Ovarian weights[2]		No.	Ovarian weights[2]	
				Range	Average		Range	Average
		mg.		*mg.*	*mg.*		*mg.*	*mg.*
Normal	43	6.4	11	15–48	23.5	1		37.0
Castrated (2–5 mo.)	14	7.6	0			7	43–94	66.2
	7	4.1	0			7	32–59	48.1
	2	2.1	0			3	27–60	41.4
		0.9	0			2	17–28	22.5
Castrated (2–5 mo.) 0.5 mg. T.P.[1] 15 days	4	1.65	0			7	43–62	52.6
Castrated (2–5 mo.) 0.5 mg. T.P.[1] 30 days	5	1.56	9	26–45	35.2	1		53.0
Castrated (2–5 mo.) 0.5 mg. T.P.[1] 45 days	3	1.6	6	18–41	30.3	0		

[1] Testosterone propionate in sesame oil.

[2] The ovaries of a group of 8 control rats hypophysectomized at 21 days of age and necropsied 7 days later averaged 8.41 mg. (range 7.94 to 9.84 mg.).

ranged from 16 to 123 mg. in the normal recipients and 17 to 94 in the hypophysectomized animals, but in all of the ovaries, regardless of size, corpora lutea were found. In a previous report (6), it was shown that the increase in the pituitary luteinizing factor becomes demonstrable about 15 days after removal of the gonads.

Effect of testosterone propionate on gonadotropic content of pituitaries of castrated rats. In this series, 15 animals, castrated 2 to 5 months previously, received daily amounts of 0.5 mg. of the androgen in 0.05 cc. of sesame oil for a period of 15 days; 15 similar animals received the same daily dose for 30 days; and a third group of 11 castrated animals was injected with 0.5 mg. of androgen for 45 days.

A daily dose of 0.5 mg. of testosterone propionate, over periods of 30 and 45 days, was found to be effective in bringing about a marked reduction of the luteinizing activity of the pituitaries of castrated rats. Suspensions of the acetone-dried pituitary powder from these donors were administered to 30 normal and 16 hypo-

physectomized recipients. Of the 30 normal test animals, no lutein tissue was found in the ovaries of 24 (table 1). The results obtained with hypophysectomized recipients were even more conclusive for, as may be seen in table 2, stimulation was limited to follicular development in the ovaries of 15 of these test rats; the remaining animal had 2 corpora lutea in one ovary. These results are in strking contrast with those obtained from the injection of the pituitaries of the untreated castrated males.

Treatment of castrated males for a period of 15 days with the same daily dose (0.5 mg.) of this androgen, is apparently insufficient to liberate the LH from the pituitaries. Suspensions of the powdered pituitaries from these animals were administered to 17 normal and 7 hypophysectomized recipients. The ovaries of the recipients, without exception, contained corpora lutea (tables 1 and 2).

In order to determine if testosterone propionate is effective in preventing the luteinizing factor from accumulating in the pituitary after removal of the testes, 12 adult males were castrated and injections of 0.3 mg. of testosterone propionate daily were started 5 days postoperatively and continued for 30 days. The autopsies were made on the day after the last injection. The pituitaries of these animals were tested in 6 normal 21-day-old recipients and, as shown in table 1, the ovaries of 5 animals contained no corpora lutea.

It is of interest to note that, although prolonged administration (30 and 45 days) of testosterone propionate depleted the luteinizing factor in the pituitary, the follicle-stimulating factor was not greatly decreased as might be expected. The equivalent of one-half of a pituitary (average 1.6 mg. of dried powder) obtained from castrated animals previously treated for 45 days with testosterone propionate stimulated the ovaries of the recipient animals in a degree comparable to that of 2 pituitaries (average 3.6 mg. dried powder) from normal adult males. Similar stimulation resulted from the administration of 0.9 mg. of the pituitary powder from castrated, untreated males.

Gonadotropic factors present in the blood serum of a) normal, b) castrated and c) castrated rats treated with testosterone propionate. The blood sera of these animals were assayed for their follicle-stimulating and luteinizing activities by indirect methods, since the amount of these factors in the serum was insufficient to be established by the usual techniques. To determine the amount of FSH in the serum, utilization was made of the observation (8), that small amounts of FSH interact with chorionic gonadotropin from pregnancy urine to produce an augmented or potentiated response in the ovaries of immature female rats. Since the chorionic gonadotropin is not potentiated by serum *per se* through the medium of delayed absorption it was felt that any potentiating effect which might be observed with the combined injections of these two substances could safely be attributed to FSH hormone in the blood serum. Accordingly each recipient received 10 cc. of rat serum, in combination with a constant amount of gonadotropin from pregnancy urine.

The chorionic gonadotropin preparation, injected alone in 14 animals at the control level used in these tests, produced ovaries weighing an average of 27 mg. Following the administration of the gonadotropin, together with 10 cc. of the blood serum from normal adult males, to each of 7 recipients, the resultant average ovarian weight was 37 mg. (table 3). On the other hand, 10 cc. of blood serum from untreated castrated male rats caused a marked potentiation of the urinary gonadotropin response. In 9 such recipients the average ovarian weight was 70.2 mg. Injections of the castrated animals with testosterone propionate for periods of 15 to 45 days, however, decreased this potentiating effect of their serum; 11 immature female rats each received 10 cc. of serum from such treated castrated males, together

with urinary gonadotropin, and the ovaries averaged 32.3 mg. (table 3). By starting the injections of testosterone propionate soon after castration it was possible to prevent the appearance of an increased amount of FSH in the blood stream. Twelve adult males received 0.3 mg. of testosterone propionate daily, from the 5th to the 35th day after removal of the testes, and the blood serum from these animals injected with the chorionic gonadotropin into 3 immature female rats produced ovaries averaging 33.6 mg.

Since the luteinizing potency of the pituitary was markedly increased after castration and since it was possible to reverse this change by the administration of

TABLE 3. AMOUNT OF FSH PRESENT IN BLOOD SERA OF *a)* NORMAL ADULT MALE RATS, *b)* CASTRATED MALES AND *c)* CASTRATED MALES TREATED WITH TESTOSTERONE PROPIONATE, AS DETERMINED BY ABILITY OF THESE SERA TO AUGMENT OVARIAN RESPONSE OF NORMAL IMMATURE FEMALE RATS TO A GIVEN DOSE OF PREGNANCY URINE GONADOTROPIN

Donors		Recipients[1]		
Type	Treatment	Number	Ovarian weights	
			Range	Average
			mg.	*mg.*
Normal		7	32–40	37.0
Castrated (2–5 mo.)		9	39–89	70.2
Castrated (2–5 mo.)	0.5 mg. T.P.[2] 15–45 days	11	21–49	32.3
Castrated (5 days)	0.3 mg. T.P.[2] 5th–35th day	3	27–40	33.6

[1] Each recipient received chorionic gonadotropin plus 10 cc. of serum. The chorionic gonadotropin administered alone at the same dose level as was used in all the assays shown in this table resulted in an average ovarian weight of 27 mg. in 14, 21-day-old, female rats.

[2] Testosterone propionate in sesame oil.

testosterone propionate, it seemed possible that detectable alterations in the amount of LH present in the blood stream might also be found. Accordingly, the serum was administered in 10 cc. total doses, with a constant amount of a follicle-stimulating extract (FSH) of sheep pituitaries to normal immature female rats. The ability of the different blood sera to alter qualitatively the effects of this FSH preparation, as shown by the incidence of corpora lutea in the recipients' ovaries, was used to indicate the relative concentration of LH in the blood serum. The ovaries of 10 immature females rats which received FSH alone at the control dose level showed follicular growth with no evidence of luteinization. The average weight of the ovaries was 24 mg. Following the administration of this FSH extract, in combination with 10 cc. of blood serum obtained from normal adult males to 7 normal immature test animals, the ovaries of 5 contained corpora lutea (table 4). On the other hand, the blood serum of castrated males, injected together with FSH, induced no lutein development in 5 of 6 test animals. But corpora lutea were present in the ovaries of 7 of 8 recipients receiving the FSH together with 10 cc. of blood serum from castrated animals which had been treated with testosterone propionate for periods of 15 to 30 days. After treatment of the donors with this androgen for a period of 45 days, the serum no longer induced lutein development when administered with FSH to 5 recipients (table 4).

DISCUSSION

The results of the present study demonstrate that the secretion of the gonads may induce qualitative alterations of the hormonal content of the anterior pituitary. The pituitary of the normal adult male rat contains primarily follicle-stimulating hormone (FSH), having relatively little capacity to stimulate the development of corpora lutea in normal or hypophysectomized immature female rats. This does not necessarily indicate that the pituitary of the normal male rat does not produce the LH factor, but more likely that this factor is readily released under the influence of testicular secretion. There is some demonstrable LH activity in the blood serum of these animals.

After removal of the gonadal influence by castration, the amount of the LH

TABLE 4. RELATIVE LUTEINIZING ACTIVITY OF SERA OF NORMAL, CASTRATED, AND TESTOSTERONE PROPIONATE TREATED-CASTRATED RATS AS DETERMINED BY INJECTING THESE SERA ALONG WITH A CONSTANT AMOUNT OF AN FSH PREPARATION INTO 21-DAY-OLD FEMALE RATS AND NOTING THE APPEARANCE OF CORPORA LUTEA IN THE OVARIES

Donors		Recipients[1]					
		Weight of pairs of ovaries showing:					
Type	Treatment	Follicles only			Corpora lutea		
		No.	Range	Average	No.	Range	Average
Normal		2	32–112	72.0	5	56–138	106.2
Castrated (2–5 mo.)		5	41–82	60.0	1		107.0
Castrated (2–5 mo.)	0.5 mg. T.P.[2] 15–30 days	1		62.0	7	64–133	93.0
Castrated (2–5 mo.)	0.5 mg. T.P.[2] 45 days	5	42–56	47.8	0		

[1] Each rat received a constant amount of FSH and 10 cc. of serum. The FSH injected alone at the same dose level as was used in these experiments produced only follicular growth. The average weight of the ovaries of 10 rats was 24 mg.

[2] Testosterone propionate in sesame oil.

factor within the pituitary is markedly increased. The pituitaries of males castrated 2 to 5 months previously induced luteinization without exception in the ovaries of immature recipients, regardless of the degree of ovarian response.

Injections of testosterone propionate prevented the LH from accumulating in the pituitaries of castrated male rats when treatment was begun soon after the operation. Furthermore, if the treatment was delayed after castration until much LH had accumulated in the pituitaries, then the injections of this androgen brought about the reduction (release) of LH to the approximate level of that found in the normal male rat pituitary.

These differences in the concentration of the pituitary gonadotropic factors cannot be demonstrated if the pituitaries are assayed by implantation of the fresh glands, as was shown previously (6). The pituitaries of castrated males which, if acetone-dried, always produce corpora lutea in immature recipients, stimulate only follicular development if implanted in the fresh state. That the implanted pituitaries retain the LH factor was demonstrated by recovering the implants and reassaying them after acetone desiccation. The failure of these glands to induce luteinization when

first implanted may be accounted for by insufficient estrogen secretion on the part of the immature recipients adequately to release the LH from the implants.

The alterations in the gonadotropic complex in the pituitary following castration are reflected by changes in the follicle-stimulating and luteinizing activities in the blood serum. Since relatively large amounts of the serum (10 cc.) were required for each test, the number of recipients was necessarily limited and the results are but suggestive. However, they are consistent with the viewpoint, gained from the pituitary assays, that testosterone increases the liberation of LH from the pituitary.

The results concerning the presence of LH in the blood stream were not clear cut. Most of the immature test rats receiving serum of normal males, or castrated males which had been injected with testosterone propionate, contained corpora lutea in their ovaries, whereas those receiving serum from untreated castrated males did not. This does not necessarily signify an absence of the luteinizing factor in the latter serum, but more probably, that the method of assay used was not sufficiently sensitive to detect it. In later experiments it has been found that the blood serum of castrated adult rats does stimulate the prostate glands of hypophysectomized immature male recipients (unpublished). This observation is consistent with the finding (9) that the prostate of the hypophysectomized immature male rat appears to be a more sensitive test organ for the action of LH (ICSH) than are the ovaries of immature female rats.

If testosterone releases LH (ICSH) from the pituitary, and this in turn stimulates interstitial tissue to a greater production of testosterone, what mechanism operates to keep the production of LH in check? The answer to this question is beyond the purpose of this investigation. We did note however that prolonged administration (45 days) of testosterone led to a decrease in the amount of LH in the pituitary as well as in the blood, indicating that the androgen may tend to suppress LH formation.

Castration led to a marked increase in the amount of FSH in the serum of rats similar to that occurring in the blood and urine of menopausal or gonadectomized human beings. However, following the administration of testosterone propionate for 15 to 45 days to castrated rats the concentration of FSH in the blood stream was greatly reduced.

The separate effects of follicle stimulation and luteinization are attributed by some investigators not to separate pituitary factors, but rather to the manner of administration, the degree of response and/or the chemical manipulation in the process of fractionation (10, 11, 12). In this study, all of the animals were of the same inbred strain; the simple chemical treatment (acetone desiccation) was identical for all types of pituitaries tested; furthermore, the manner of administering the preparation and examination of the ovaries was constant for all groups. Nevertheless, the separate FSH and LH effects were clearly discernible. The alterations of the pituitary gonadotropic complex took place within the living animal and were physiological in nature. Furthermore, the manifestation of luteinization was not dependent upon the degree of ovarian stimulation. The pituitaries of untreated castrated males induced luteinization, without exception, although the dosages given were chosen to produce a wide range of ovarian stimulation (16 to 133 mg.). On the other hand, the pituitaries of normal males and castrated males treated for 30 to 45 days with testosterone propionate, produced pairs of ovaries weighing from 20 to 70 mg., of which 82 per cent contained no corpora lutea.

SUMMARY

Determinations were made of the relative content of follicle-stimulating (FSH) and luteinizing (LH) (ICSH) factors in the pituitaries and blood serum of 71 normal

adult male rats, 42 castrated untreated adults and 53 castrated males which had been treated for periods of 15 to 45 days with testosterone propionate. The results indicate that the internal secretion of the testes brings about the release of the luteinizing factor from the pituitary, and suppresses the liberation of the follicle-stimulating hormone.

The pituitaries of normal male rats contain primarily follicle-stimulating hormone while the blood serum of these animals contains luteinizing hormone, and small amounts of follicle-stimulating hormone. Following castration the luteinizing hormone, as well as follicle-stimulating hormone, are markedly increased in the pituitary; the follicle-stimulating hormone in the blood stream is also present in greater amounts, whereas the luteinizing hormone appears to be decreased.

Treatment of castrated rats with testosterone propionate reduced the luteinizing hormone content of the pituitary, but had relatively little effect on the follicle-stimulating hormone. In the blood serum of these animals, the amount of follicle-stimulating hormone was lowered, whereas the luteinizing hormone appeared to be increased after 15 and 30 days of treatment but not after 45 days of treatment.

REFERENCES

1. VAN DYKE, H. B.: The Physiology and Pharmacology of the Pituitary Body. The Univ. of Chicago Press, Chicago, 1939. Vol. 2.
2. ALLEN, EDGAR, ED.: Sex and Internal Secretions. The Williams and Wilkins Co., Baltimore, 1939.
3. FEVOLD, H. L., F. L. HISAW AND R. O. GREEP: *Am. J. Physiol.* 114: 508. 1936.
4. KORENCHEVSKY, V., M. DENNISON AND K. HALL: *Biochem. J.* 31: 780. 1937.
5. WOLFE, J. M., AND J. B. HAMILTON: *Proc. Soc. Exper. Biol. & Med.* 37: 189. 1937.
6. HELLBAUM, A. A., AND R. O. GREEP: *Am. J. Anat.* 67: 287. 1940.
7. HELLBAUM, A. A., AND R. O. GREEP: *Proc. Soc. Exper. Biol. Med.* 38: 902, 1938.
8. FEVOLD, H. L., AND F. L. HISAW: *Am. J. Physiol.* 109: 655. 1934.
9. GREEP, R. O., H. B. VAN DYKE AND B. F. CHOW: *Proc. Soc. Exper. Biol. & Med.* 46: 644. 1941.
10. SAUNDERS, F. J., AND H. H. COLE: *Endocrinology* 23: 302. 1938.
11. BISCHOFF, F.: *J. Biol. Chem.* 133: 621. 1940.
12. SMITH, P. E.: Univ. of Pennsylvania Bicentennial Conference, 19–31. 1941.

SUGAR CONTENT OF BLOOD, LYMPH AND ARTIFICIAL PERITONEAL FLUID FOLLOWING INJECTION OF INSULIN*

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LITTLE direct information is available regarding the effect of insulin upon the concentration of glucose in tissue fluids. The present experiments were undertaken for the purpose of obtaining data bearing upon this point.

MATERIAL AND METHODS

In the first series of 18 experiments (8 dogs) an artificial peritoneal fluid was produced (1, 2, 3) by instilling Tyrode's solution (50 cc. per kg.) into the peritoneal cavity of trained, unanesthetized dogs, maintained on a diet of fresh scrap meat and kennel ration. Insulin was administered subcutaneously or intravenously in doses of 1 to 5 units per kilogram of body weight. Blood was obtained from the femoral artery, and peritoneal fluid by paracentesis before and at hourly intervals after injection of insulin; the animals were under constant observation for manifestations of hypoglycemia. Similar studies were performed upon 6 rats, blood being obtained from the tail.

A second series of 7 experiments was performed upon 4 dogs, anesthetized with nembutal, in which the lymphatics of a hind limb were cannulated according to the method of Drinker (4). After cannulation, lymph flow was maintained by gentle massage of the extremity, samples of lymph and femoral artery blood being withdrawn before, and at regular intervals after, injection of insulin. Glucose was determined by the method of Benedict (5).

RESULTS AND DISCUSSION

The data are presented in detail in tables 1 and 2. As noted by others (6), there was no appreciable difference in the blood-sugar response to doses of insulin ranging from 1 to 3 U per kg., nor to subcutaneous or intravenous routes of administration. A more rapid hypoglycemic response appeared to be produced with the dosage of 5 U, but too few data were obtained to permit any definite statement in this connection. Whereas the blood-sugar concentration usually fell to 30 to 40 mg. per cent during the first 2 hours, remaining at these or slightly lower levels during the succeeding 2 to 4 hours (4–6 hours after insulin), in all but one instance (*experiment 13*) the level of the peritoneal-fluid sugar fell but slightly during the first 2 hours (to about 80 mg.%), then decreased more rapidly to reach the approximate level of the blood-sugar about 4.5 to 5.5 hours after injection of insulin. Mild manifestations of hypoglycemia, such as drowsiness and weakness of the hind limbs, were observed as early as ½ to 1

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hour after administration of insulin, but convulsions and coma developed only after the glucose content of the peritoneal fluid had fallen to that of the blood, although in several instances the latter had been at the same low level for 2 to 3 hours. However,

TABLE 1. LEVELS OF GLUCOSE IN BLOOD AND PERITONEAL FLUID OF DOGS

Experiment No.	Blood Glucose, mg./100 cc.							Peritoneal-Fliud Glucose, mg. per 100 cc.							Dosage, Comment
	Before insulin	1	2	3	4	5	6	Before insulin	1	2	3	4	5	6	
		hours after insulin							hours after insulin						
1	91	[1]45	33	26				104	[1]107	99	88				1 U/kg. subcutaneously
2	103	[1]61	45	41	34			100	[1] 91	86	65	43	0		1.5 U/kg.; convulsions at 5 hours
3	95	[1]66	52	33				101	[1] 95	86	62				2 U/kg. subcutaneously died accidentally
4	83	[1]71	46	37	34	26		98	[1] 91	84	83	66	41		2 U/kg. subcutaneously
5	93	[1]54	41	35	31	32		99	[1] 86	81	73	55	31		3 U/kg. subcutaneously convulsions at 5 hours
6	104	[1]61	42	39	39		29	97	[1] 88	85	78	58		30	3U/kg. subcutaneously
7	82	[1]55	49					97	[1] 87	85					1.2 U/kg. intravenously
8	74	[1]46	45		34	35	43	96	[1] 81	79		58	42	39	2 U/kg. intravenously
9	79	[1]66	44	[1]42	42			98	[1] 91	71[1]	56	40			2 U/kg. intravenously twice
10	73	[1]50	53[1]	53	50			96	[1]	90[1]	57	53			2 U/kg. intravenously twice; observed up to 6 hours
11	80	[1]56	77[1]	64	51			87	[1]	84[1]	82	64			2 U/kg. intravenously twice
12	75	[1]37	69?	44	73?			87	[1] 93	105	85	86			2 U/kg. intravenously
13		[1]	56		46		35	123	[1]	55		39		46	5 U/kg. subcutaneously convulsions at 2 hours and 6 hours
14		[1]				23		81	[1]			24			5 U/kg. subcutaneously convulsions at 5 hours
15		[1]		30		27		88	[1]		45		24		5 U/kg. subcutaneously convulsions at 5 hours
16		[1]		29		31	24	88	[1]		40		23	23	5 U/kg. subcutaneously at 5 hours deeply somnolent but not comatose, 'walking movements'
17		[1]		[1]		62	41	76	[1]			[1]	31	17	3 U/kg. subcutaneously twice, convulsions at 5 and 6 hours
18		[1]		[1]		32	39	76	[1]			[1]	44	21	3 U/kg. twice subcutaneously, stuporous but not comatose at 5 hours

[1] Indicates time of insulin injection.

some dogs failed to develop coma and convulsions in the presence of low blood-glucose and peritoneal-fluid-glucose concentrations.

In one dog (*experiment* 13) the glucose content of the peritoneal fluid was 55 mg. per cent as early as 2 hours after insulin injection (5 U/kg. subcutaneously), the blood sugar at this time being 56 mg. per cent. At this time, 2 hours after insulin injection, the dog went into convulsions. Both the drop of the level of peritoneal-fluid glucose

and the onset of convulsions occurred at the same time and much earlier than in the other experiments.

The occurrence of these symptoms at the time at which a low concentration of glucose is attained in the peritoneal fluid may be purely coincidental. It is possible that both the establishment of equilibrium between the blood sugar and peritoneal-fluid sugar and the progression of cerebral changes to the point of development of convulsions or coma may require several hours without being causally related. However, the results of the experiments upon rats tend to support the hypothesis of such a causal relationship, since coma, convulsions and attainment of glucose equilibrium between the hypoglycemic blood and the peritoneal fluid occurred between 1 and 2.5 hours after injection of insulin in 4 of 6 cases.

The validity of the use, in this relation, of data obtained by the method of producing artificial peritoneal fluid depends upon the assumption that changes in the

TABLE 2. GLUCOSE LEVELS OF BLOOD, LYMPH AND PERITONEAL FLUID OF DOGS FOLLOWING INSULIN ADMINISTRATION

Experiment Number	Blood Glucose, mg./100 cc.						Lymph Glucose, mg./100						Peritoneal Fluid Glucose, mg./100 cc.				Dosage, Comment
	Before insulin	0.5	1	1.5	2	2.5	Before insulin	0.5	1	1.5	2	2.5	Before insulin	0.5	1	1.5	
			hours after insulin						hours after insulin						hours after insulin		
19	54	[1]				12.6			1			52					3 u/kg. subcutaneously
20		1		32		12.6			1	46.6	31.5						3 u/kg. subcutaneously
21	55	1	34		26				1	50	38	33					3 u/kg. subcutaneously
22	84	1	43		38	28.0			1	42		34	29				4 u/kg. subcutaneously
23	75	[1]44	32	21					[1]75	36	35			88	1 41	20	20	4 u/kg. subcutaneously
24	93	[1]56	39	36			108	[1]99	44	35				83	1 89	65	65	3 u/kg. subcutaneously
25	104	[1]57	39	31			107	[1]81	59	34				104	[1]103	107	93	2 u/kg. subcutaneously

[1] Indicates time of insulin injection.

composition of this fluid correctly mirror those in normal extracellular fluid. Although this assumption has been supported, to some extent at least, by previous studies (1, 2, 3), practically all of these have been concerned with the establishment of equilibrium between the blood and peritoneal fluid in the presence of higher values in the former than in the latter. Obviously, there may be a marked difference in the rate of passage of solutes in the opposite direction under different conditions. The glucose content of lymph was determined in order to secure what may be more direct information regarding its concentration in normal tissue fluid than can be obtained by the method of artificial peritoneal fluid. It was found that the fall in the level of lymph sugar lagged somewhat behind that in blood sugar in 6 of the 7 experiments. Equilibrium between the two was established at about 1.5 hours after injection of insulin in 3 instances; in the other 3, the concentration in the lymph was considerably higher than in the blood after 1.5, 2 and 2.5 hours, respectively. The hypoglycemic response to insulin was more pronounced in the anesthetized than in the unanesthetized animals, a one-hour drop of about 55 per cent below the control level (to 35.5 ± 3.1 mg.%) occurring in the former as compared to 37 per cent (to 60.8 ± 9.8 mg.%) in the latter. Moreover, in 2 of the 3 anesthetized dogs in which glucose was determined simultaneously in the blood, lymph and peritoneal fluid, its concentration in the latter fell much more rapidly than in the unanesthetized animals, equilibrium being established 1 hour after injection of insulin in one case. In the third dog, the findings were similar to those obtained in unanesthetized animals. Heim and Berg (6) reported similar observations in one diabetic andtwo normal dogs. The lymph and blood-sugar curves paralleled one another more closely than those in the present study, but their

animals were unanesthetized and the insulin dosage was much smaller than that employed by us.

The lack of consistent relationship between the time of occurrence of severe 'hypoglycemic' manifestations such as convulsions and coma, and the degree of hypoglycemia may be due to a lack of consistent quantitative relationship between the latter and the concentration of glucose in the interstitial fluid of the tissue (brain) concerned with the development of these phenomena.

The relation of the occurrence of coma and convulsions to the glucose content of the peritoneal fluid suggests that the latter may be representative of normal interstitial fluid glucose concentration. The fact that changes in the lymph of the limb can occur more rapidly than in the peritoneal fluid in the presence of falling blood-sugar levels may be due to some anatomical relationship between certain blood and lymph capillaries which permits equilibrium between these two fluids to be established more quickly than if it had to be mediated by a relatively large volume of interstitial fluid. The possibility must also be considered that the rapidity and magnitude of changes in the glucose content of the interstitial fluid of the brain differ from those in the interstitial fluid or the lymph of the hind limb.

SUMMARY AND CONCLUSIONS

1. Hypoglycemic convulsions and coma occur 4 to 6 hours after subcutaneous or intravenous insulin injection into the dog.

2. The blood sugar reaches hypoglycemic levels much earlier, usually within 2 hours after insulin injection.

3. The glucose content of an originally isoglycemic artificial peritoneal fluid (Tyrode's solution) decreases more slowly, equilibrium with the hypoglycemic level of blood sugar being established 4 to 6 hours after insulin. If consulsions and coma occur, they appear at this time. Some dogs fail to develop convulsions and coma in spite of low sugar concentrations in the peritoneal fluid and blood.

4. In rats, convulsions and coma occur 1.5 to 2 hours following administration of insulin, the glucose content of the artificial peritoneal fluid reaching low levels at this time.

5. If the changes in the artificial peritoneal fluid reflect accurately changes in the extracellular tissue fluids, the onset of convulsions and coma in the dog several hours after the blood sugar has reached its minimum concentration is adequately explained. However, there is no apparent explanation for the absence of convulsions or coma in some cases, despite the presence of low blood sugar and peritoneal-fluid sugar concentrations.

6. In dogs anesthetized with nembutal the blood sugar level drops more rapidly after administration of insulin than in the unanesthetized animal. The glucose content of the artificial peritoneal fluid also decreases more rapidly than in the unanesthetized dog.

7. The decrease in lymph sugar lags considerably behind that in blood sugar during the first hour following insulin administration in the anesthetized dog, but the lymph reaches equilibrium with the blood in this respect more rapidly than does the peritoneal fluid.

8. The question is briefly discussed as to whether the changes in the artificial peritoneal fluid or those in the lymph of the extremities are the more nearly representative of changes in normal extracellular fluid.

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REFERENCES

1. Schechter, A. J., M. K. Cary, A. Carpentieri and D. C. Darrow: *Am. J. Dis. Child.* 46: 1015. 1933.
2. Cantarow, A. and V. Haury: *Am. J. Physiol.* 126: 66. 1939.
3. Cantarow, A., and A. Rakoff: *Endocrinology* 27: 652. 1940.
4. Drinker, C. K., and J. M. Yoffey: Lymphatics, Lymph and Lymphoid Tissue. Harvard University Press, Cambridge, 1941.
5. Hawk, P. H., and O. Bergeim: Practical Physiological Chemistry. 11th ed. P. Blakiston Sons, Philadelphia, p. 436.
6. Zucker, T. F., and B. H. Berg: *Am. J. Physiol.* 119: 531. 1937.
7. Heim, J. W., and B. H. Berg: *Am. J. Physiol.* 105: 674. 1933.

STUDY OF EXCRETION OF PITUITARY HORMONES IN HUMAN URINE

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IN THE PAST few years great strides have been made in the isolation and purification of hormones from crude animal pituitary extracts (1–6). Satisfactory methods have been devised for testing minute quantities of these substances. Little has been done, however, with material from human sources since Smith (7) in 1934 demonstrated on hypophysectomized animals that the active protein in the urine of castrated and menopausal subjects was similar to the pituitary follicle-stimulating hormone.

It seemed that the methods developed for investigating crude pituitary extracts might, with slight modifications, be adapted to the study of human urinary proteins and their biological activities.

METHODS

Large volumes of urine were collected, using approximately 2.5 gm. of sulfanilamide powder per liter to prevent bacterial growth and refrigerating the urine as soon as possible after collection to avoid destruction of any heat-labile protein.

Step 1. The urine was filtered rapidly, placed in dialysis bags approximately 4 feet long and one inch in diameter, and hung in a cold room at 10 C. to dialyze. By this method concentration of the urine was effected simultaneously with dialysis. When the bags became encrusted with salts, the non-dialyzable residues were combined and placed in new dialysis tubing maintaining the 4 feet of pressure and thus facilitating dialysis and concentration. After about 2 weeks sufficient concentration was obtained. The urine was then dialyzed against cold running water to remove the remaining sulfanilamide, urea and salts. Care was taken not to concentrate below one-tenth of the original volume lest the urea content become great enough to denature the proteins. The final concentrate was filtered by suction with Kieselguhr.

Step 2. The hydrogen-ion concentration was adjusted to pH 4 by means of the glass electrode and the heavy precipitate which formed was filtered off and saved for bioassay.

Step 3. The filtrate was divided into 3 or 5 equal amounts which were then saturated with ammonium sulfate and readjusted to various pH values. The optimum was found to be about pH 6.8 and this was adopted as the standard value for precipitation. The precipitates which formed after the solutions were allowed to stand in the cold 48 hours were filtered off. These precipitates were taken up in water with the addition of a small amount of ammonium hydroxide to increase the solubility and saturated again with ammonium sulfate. The pH values were readjusted to their respective previous readings and the resulting precipitates filtered off as before. This procedure was repeated from 4 to 6 times for further purification. The final precipitate

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was dissolved in a slightly alkaline solution and dialyzed against cold running water for 12 hours. The solution was then frozen and kept in this form until bio-assay could be performed. All of the filtrates were saved, dialyzed, re-concentrated and tested for biological activities.

Various steps in the procedure were tested for losses. In *step* 1 no appreciable loss of gonadotropic activity was incurred by the concentration and dialysis. This was determined by comparing an alcohol precipitate of the original urine with an alcohol precipitate of a calculated volume equivalent of the dialyzed final concentrate.

The precipitate obtained in *step* 2 when the concentrate was adjusted to pH 4, was found to contain no appreciable gonadotropic activity. A calculated volume of concentrate, equivalent to 2340 cc. of urine was assayed and found to have no biologic activity.

When full saturation with ammonium sulfate was used in *step* 3 at a favorable pH for precipitation and further purification of the protein, no appreciable loss of gonadotropic activity occurred. When, however, one-half or one-fourth saturation of ammonium sulfate was used gonadotropic activity was found in the filtrates. This latter method of purification was, therefore, abandoned and only full saturation was used.

MATERIAL

The material studied was urine from menopausal women, women with normal menstrual cycles and urine of normal men.

Bio-assays. The test animal was the immature, hypophysectomized male rat. Animals were hypophysectomized on the 24th to the 26th day of life and assays were begun 7 days later. Injections were given twice daily over a 7-day period. The animals were killed and the organs weighed on a torsion balance. Sections were taken for microscopic examination. Preliminary tests, in order to judge the approximate gonadotropic activities of the preparations, were made on normal immature female rats using ovarian and uterine weights as the biologic end points.

EXPERIMENTS

Substances with biologic activity resembling that of the follicle-stimulating hormone of the anterior pituitary are the only ones which have been found consistently in the urine by numerous observers. Menopausal urine is known to furnish a potent source for this material. It was therefore decided to use the activity of this hormone as a guide for the efficacy of the method. Menopausal urine was fractionated by the method previously outlined and the fractions were tested on normal immature female rats. In this way it was determined that the optimum pH range for the precipitation of the active gonadotropic substance was between pH 5 and 7. This was then checked on hypophysectomized male animals and comparable values were obtained (table 1).

The data in table 1 show no evidence of any biological activity other than that resembling the follicle-stimulating hormone except possibly in the fraction precipitated at pH 6 in *Experiment I.* In this the ventral prostatic weight of 10.5 mg. is slightly excessive suggesting activity of the luteinizing hormone[1] (8), but as the number of animals tested was necessarily small and as the microscopic examination of the

[1] The term luteinizing hormone (LH) has been used as synonymous with interstitial-cell-stimulating hormone (ICSH).

testicles showed no evidence of interstitial cell stimulation, this value was not interpreted as meaning that LH was present.

Having determined the optimum pH value for the precipitation of the active gonadotropic substance, a large volume of menopausal urine was precipitated at pH 6.8 after having been saturated with ammonium sulfate as previously described. Following purification, the fraction was assayed. The average weights and the percentage body weights of the various organs can be seen in table 2. Again there was no evidence of any activity but that attributable to the follicle-stimulating hormone.

As this finding seemed to confirm the original work of Smith (7) showing that urines of menopausal and castrated subjects contain pure FSH we next tried to secure a source which might be expected to contain the luteinizing hormone. Since Shedlovsky et al. (2) have said this substance produces ovulation and also causes estrogen

TABLE 1. EFFECT OF VARIATIONS IN HYDROGEN ION CONCENTRATION ON THE PRECIPITATION OF A FOLLICLE-STIMULATING SUBSTANCE FROM MENOPAUSAL URINE

pH	Average Weight in mg.					Equivalent Volume of Urine Tested cc.
	Testes	Ventral Prostate	Seminal Vesicles	Adrenal	Thyroid	
			Experiment I			
6	227.5	10.5	7.0	5.1	4.2	440
5	155.2	7.2	6.0	4.0	4.0	440
4	138.0	6.9	6.1	4.2	3.8	440
3	107.0	7.0	5.0	3.9	3.0	440
2.7	98.0	5.0	6.8	5.4	4.8	440
			Experiment II			
7	191.5	6.0	5.1	4.0	3.0	200
4	164.0	5.0	5.2	4.0	3.5	200
2.7	82.0	4.0	5.1	3.0	3.0	200

secretion from the maturing Graafian follicle, it seemed that the urine from normally menstruating women might be an adequate source for LH. Therefore urine was collected, under conditions outlined above, throughout the entire menstrual cycle from two subjects who had had consistently regular menses accompanied by breast symptoms indicative of luteal function. As a further check on ovulation the dialysate from *step 1* was saved during the last half of the collection period and pregnanediol determinations were made (9). Both specimens contained appreciable amounts of free pregnanediol indicating that ovulation and corpus luteum formation in both subjects had occurred. The menstrual cycles were divided into 4 parts of 7 days each and the urine collected in each of these periods was fractionated, purified and tested separately. Follicle-stimulating activity was found in the fraction which precipitated at pH 6.8 after saturation with ammonium sulfate. This activity was found in urine obtained throughout the cycle. No evidence of LH activity was observed as judged by the weights of the ventral prostate and the seminal vesicles, or by histological examination for interstitial cell proliferation. Neither of the precipitates which occurred at pH 4 (*step 2*), nor the filtrates from the precipitates at pH 6.8 (*step 3*) showed any biological activity. There was no evidence of adrenotropic activity, as judged by the adrenal weight, or of thyrotropic activity, as judged by the height of the thyroid epithelium on microscopic examination (table 2).

It was thought that the treatment of the filtrates (*step* 3), might have been such as to destroy the LH activity. LH isolated from hog pituitaries by Chow, et al. (5) has an iso-electric point of about pH 7.45 while that isolated from sheep by Li, et al. (3) has an iso-electric point at about pH 4.8. Thus there is apparently a wide species variation. It was therefore thought advisable to precipitate some specimens at a different pH value. Two specimens of urine were collected from regularly menstruating women over a 7-day period from the 7th to the 14th day of the cycle. These samples were treated exactly as the previous ones except that after saturation with ammonium sulfate, precipitation was effected at pH 4 instead of at pH 6.8. Neither specimen showed any LH activity (table 2).

TABLE 2. EFFECTS OF VARIOUS URINARY PROTEIN FRACTIONS ON ORGAN WEIGHTS OF HYPOPHYSECTOMIZED IMMATURE MALE RATS[1]

Source of Material	Testes		Ventral Prostate		Seminal Vesicles		Adrenals		Thyroid	
	Average mg. wt.	% Body wt.	Average mg. wt.	% Body wt.	Average mg. wt.	% Body wt.	Average mg. wt.	% Body wt.	Average mg. wt.	% Body wt.
Controls	74.8	0.14	4.9	0.009	5.4	0.010	4.7	0.009	3.9	0.007
Menopause ppt. pH 6.8	154.3	0.38	5.9	0.014	4.7	0.011	4.6	0.011	3.9	0.009
Normal female ppt. pH 6.8										
No. 1[2]	171.0	0.34	6.4	0.013	6.1	0.012	5.6	0.012	4.2	0.008
No. 2[2]	123.4	0.30	5.3	0.013	5.9	0.014	4.7	0.011	4.0	0.009
No. 3[3]	137.0	0.25	6.0	0.011	5.8	0.010	6.2	0.010	4.5	0.008
ppt. pH 4 No. 4[3]	69.6	0.15	3.7	0.008	4.6	0.010	4.2	0.009	3.9	0.008
Normal male ppt. pH 4	133.0	0.27	5.7	0.011	5.0	0.010	5.9	0.012	3.8	0.007

[1] No basis for a quantitative comparison of these results has been included as in many instances the work has been only qualitative and any conclusions as to the relative amounts of hormone present in the source materials would be erroneous.

[2] All fractions of menstrual cycle totaled.

[3] Only urine for 7 days from 7th to 14th day of menstrual cycle.

Another possible source of LH seemed to be urine of normal men. This was consequently studied. Once again precipitation was effected at pH 4. As shown in table 2, FSH activity was obtained, but no LH.

SUMMARY

An attempt has been made to concentrate and fractionate human urine in such a way that the proteins will not be destroyed or changed. These fractions have been tested on hypophysectomized animals for various pituitary hormone activities. Urine from menopausal women, normally menstruating women and normal men has been used.

A biological activity which corresponds to that of the follicle-stimulating hormone of the anterior pituitary has been found in all types of specimens examined. This active fraction is best precipitated from a saturated solution of ammonium sulfate at pH 6.8.

No evidence of LH, thyrotropic or adrenotropic activity has been found in any fraction of any specimen studied.

REFERENCES

1. Chow, B. F., R. O. Greep and H. B. van Dyke: *J. of Endocrinology* 1: 439. 1939.
2. Shedlovsky, T., A. Rothen, R. O. Greep, H. B. van Dyke and B. F. Chow: *Science* 92: 178. 1940.
3. Li, C. H., M. E. Simpson and H. M. Evans: *Endocrinology* 27: 803. 1940.
4. Greep, R. O., H. B. van Dyke and B. F. Chow: *Endocrinology* 30: 635. 1942.
5. Chow, B. F., H. B. van Dyke and R. O. Greep: *Endocrinology* 30: 650. 1942.
6. Chow, B. F.: *Endocrinology* 30: 657. 1942.
7. Smith, P. E., E. T. Engle and H. H. Tyndale: *Proc. Soc. Exper. Biol. & Med.* 31: 745. 1934.
8. Greep, R. O., H. B. van Dyke and B. F. Chow: *Proc. Soc. Exper. Biol. & Med.* 46: 644. 1941.
9. Astwood, E. B., and G. E. Seegar Jones: *J. Biol. Chem.* 137: 397. 1941.

METHODS OF ADMINISTERING DESOXYCORTICOSTERONE AND THE PROBLEM OF ITS INACTIVATION BY THE LIVER[1]

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WE HAVE previously presented evidence indicating that the natural secretions of the adrenal cortex of the rat, unlike those of the gonad, are not inactivated to any significant extent by the liver(1). This was concluded from experiments testing the functional capacity of regenerated adrenal cortex tissue growing on the intestinal mesentery as compared to that at other transplant sites.

The possibility, however, that the synthetic substance, desoxycorticosterone acetate, is inactivated by the gut or its derivatives was suggested by the observation that this material, unlike other crystalline cortical substances, was inactive when given in single daily doses by stomach tube (2, 3), and that this inactivity was not due to the excretion of the material in feces (2).

To test more specifically the role of the liver in this inactivation three desoxycorticosterone acetate-cholesterol[2] pellets were implanted in *a*) the spleens, *b*) the kidneys, or *c*) subcutaneous tissues of young adrenalectomized rats (4). It was noted that pellets in the spleen, presumably with hepatic portal drainage, maintained life and growth as well as those located in the kidneys; life, if not growth, was maintained as well as by those placed subcutaneously. These observations were recorded in an abstract (4); and the complete data on which they were based are given here (fig. 1, ser. 1, 4, 6).

Recently, however, Selye (5) showed that the anesthetic effect of desoxycorticosterone acetate was much greater in the absence of the liver; and Burrill and Greene (6) noted quantitative reduction in the effectiveness of pellets of pure desoxycorticosterone acetate when absorption took place from mesenteric pockets as compared to subcutaneous sites. In contrast, Grollman has long commended the oral administration of cortical substances, including desoxycorticosterone acetate (7), as being a highly effective method of administration in rats.

In an attempt to correlate these apparently incompatible findings, we have extended our earlier experiments and used a wide variety of implantation sites for pellets, as well as different feeding techniques for administration of desoxycorticosterone acetate. In general our experiments confirm, insofar as they duplicate, those of Burrill and Greene.

METHODS

Pellets were prepared in an apparatus combining some features of those described

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[2] The desoxycorticosterone acetate (Cortate) used was supplied by Dr. Erwin Schwenk of the Schering Corp. Inc., Bloomfield, N. J.

by Mark and Biskind (8) and Hartman (9). To avoid fragility of the pellets cholesterol was added to the desoxycorticosterone acetate. This was done as follows. Two parts of cholestreol and 1 part of desoxycorticosterone acetate were dissolved in absolute alcohol. The material was then evaporated to dryness and the crystals made into pellets, a procedure copied in part from that of Ingle and Mason (10). The resulting pellets were small, averaging 2.27 mg., of which two-thirds was cholesterol. One or three pellets were implanted, at the time of adrenalectomy, into male male rats weighing from 50 to 60 gm. The stock diet was that previously described (11). For every

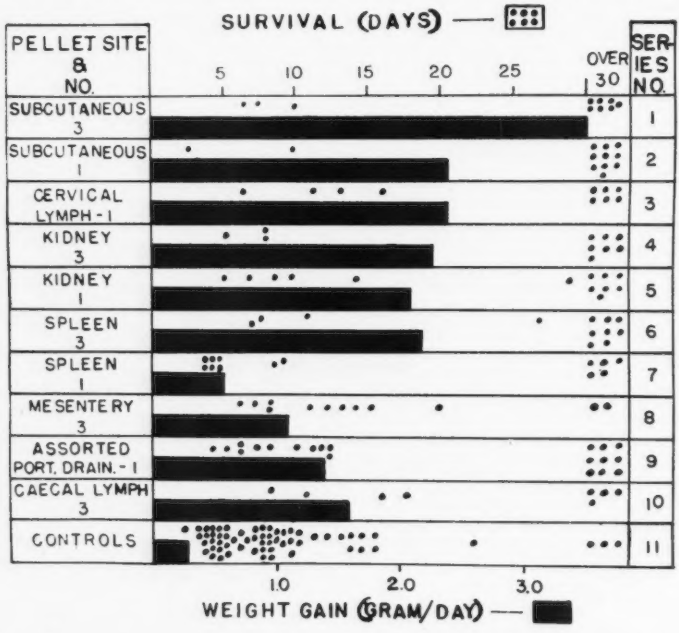

Fig. 1. Survival and growth rate of adrenalectomized rats having either one or three desoxycorticosterone acetate-cholesterol pellets implanted at various sites. Each dot represents an individual animal.

series of treated animals a group of controls, either untreated, or bearing cholesterol pellets at the sites mentioned below, were observed. Since the cholesterol was without effect the data on all controls were combined (fig. 1). Animals showing any gross cortical tissue at autopsy were discarded.

For implantation, several sites with hepatic portal drainage were used. At first pellets were placed in the medial surface of the spleen—this surface being chosen in order to minimize adhesions to the body wall. Such adhesions were frequent nevertheless but they could be easily detected. A complication more difficult to control and detect was noted in some of the later cases, namely, the occurrence of anastomoses between branches of the severed left adrenal vein and the spleen. Pellets were also easily placed in the cavity of the greater omentum in the gastrosplenic region, but these too often formed circulatory connections with the adrenal veins. Pellets placed in mesenteric pockets as described by Burrill and Greene (6) were free of these complications and probably had solely hepatic portal drainage. Lymph

glands in the region of the caecum afforded a convenient implantation site, but one with the possibility of slight drainage into the vena cava.

Pellets were placed subcutaneously in the ventral neck region just anterior to the sternum. Through a higher incision pellets were inserted directly into cervical lymph glands. Pellet implants into the kidney were made through the incision for removal of the left adrenal, and placed just beneath the renal capsule. In some cases attempts were made, without consistent success, to place pellets at the site of the extirpated left adrenal. Others were dropped loosely into the body cavity and allowed to attach wherever they might; generally this was to the mesentery or omentum.

In the feeding experiments, desoxycorticosterone acetate in absolute alcohol (1 mg. in 10 cc. of alcohol) was added to the food and the mass then evaporated to dryness. The concentration of desoxycorticosterone acetate in the food was adjusted daily, if necessary, in order to keep the consumption at desired levels. Because of some variability in appetite, and hence of food intake, it was necessary to keep these animals in individual cages.

In addition, some rats received desoxycorticosterone acetate in sesame oil daily by stomach tube, and controls received sesame oil alone. Results in these animals were compared with those in animals receiving desoxycorticosterone acetate by subcutaneous injection.

RESULTS

Fate of pellets. When the pellets were allowed to remain in the animals for two months, or longer, they were found to have become soft disorganized masses which could not be removed intact from the encapsulating tissue. After one month, or less, they were still firm and easily recovered. Since the pellets were small and could not be entirely freed of all encapsulating tissue, no consistent or significant weight loss was found when they were removed after 30 days and dried. This apparent lack of appreciable absorption of pellets made of a mixture of desoxycorticosterone acetate and cholesterol can be correlated with the work of other investigators on the non-absorption of cholesterol (15) and a reduced absorption rate of other compounds when combined with cholesterol in pellet form (14).

Results with pellets. Most of the results are summarized in figure 1. The column marked 'over 30' days includes data on animals living from 31 days to 3 months. Most of the animals which survived the first 2 weeks lived from 45 to 60 days, but to recover the pellets it was necessary to kill some animals after 30 days. The figures for gain in weight are for the first 30 days only, or for the duration of life, if that was less than 30 days.

It is obvious that all pellets, wherever placed, were effective. Subcutaneous ones, and those placed in the cervical lymph nodes gave the best results (fig. 1, ser. 1, 2, 3). The latter implant site was used as a control for pellets placed in mesenteric lymph nodes. The kidney, one of the best sites for transplants of cortical tissue (1), was probably somewhat inferior to the lymph nodes as a site for pellet implants (fig. 1, ser. 4, 5). The efficacy of pellets so placed as to have hepatic portal drainage (fig. 1, ser. 6, 7, 8, 9, 10) was inferior to that of pellets in other sites, with the possible exception of *series* 6 in which 3 pellets were implanted in the spleen. The greater effectiveness of 3 pellets in the spleen as compared to that of others with hepatic portal drainage was possibly due to a more complete absorption, or to the aforementioned possibilities of collateral circulation—although attempts were made to exclude the cases in which collateral circulation was indicated. In any case, the effectiveness of one pellet in the spleen was decidedly inferior to those at sites without portal connections. Pellets placed in caecal lymph glands were somewhat more effective than those in mesenteric

pockets—again possibly because the drainage was not entirely hepatic; they were clearly less effective than those with no hepatic portal supply.

Of the pellets dropped loosely into the abdominal cavity, many adhered to mesenteric tissue at sites ranging from the greater omentum to the mesocolon. Some were attached by long strands of tissue to the mesentery. These all had in common a primary, if not complete, portal drainage and are included (ser. 9) to show again the lower effectiveness under such circumstances.

Pellets which were dropped loosely into the abdominal cavity and became adherent to the ventral peritoneal wall were relatively ineffective, perhaps because of poor vascular connections. If pellets can be so placed as to implant themselves in the region of the excised left adrenal, vascular connections are made with the adrenal vein, and probably better results than at any other site can be obtained. It proved

TABLE 1. AVERAGE FIGURES SHOWING RESPONSE OF ADRENALECTOMIZED RATS TO DESOXYCORTICOSTERONE ACETATE GIVEN BY VARIOUS MEANS DAILY FOR 10 DAYS

Treatment	Number Animals Used	Daily Food Consumption	Weight Change During Treatment	Survival	Number Animals Died During Treatment	Initial Weight
		gm.	*gm./day*	*days*		*gm.*
0.2 mg., food	18	7.1	+1.8	15.5	3	56.8
0.2 mg., injected	11	9.3	+3.4	23.0	1	54.8
0.5 mg., stomach tube	9		−0.06	6.0	9	56.9
0.05 mg., injected	8		+2.0	19.0	1	55.6

to be very difficult, however, to anchor the pellets at this site, and adequate data are lacking since most of the cases were obtained by accident.

Oral administration of desoxycorticosterone acetate. In animals fed desoxycorticosterone acetate, a dose of 0.2 mg. daily was selected, after preliminary experimentation, as suitable for comparison with a similar dose injected once daily in oil. If smaller dosages were used many of the fed animals died and their failing appetites made it difficult to maintain a constant intake. The actual dose in the animals that were fed ranged from 0.18 to 0.24 mg. per day, as the consumption was not accurately predictable. The average survival of the animals receiving desoxycorticosterone acetate in their food was less than that of those receiving similar amounts by injection; their growth rate, a more important figure, during the 10 days of treatment was only slightly more than half that of the injected animals. Food intake was also slightly less in the series receiving desoxycorticosterone acetate by mouth (table 1).

In another series of animals, desoxycorticosterone acetate was given at 0.2 and 0.5-mg. dosage levels in 0.2 cc. of sesame oil once daily by stomach tube. The substance was clearly inactive by this method of administration. The figures for the 0.5-mg. dosage are shown in table 1. In contrast, either one-tenth of that dose given patenterally or four-tenths of it in the food was clearly beneficial.

DISCUSSION

Is is apparent that pellets of desoxycorticosterone acetate so placed as to have hepatic portal drainage, are in general less effective than those placed elsewhere. This probably indicates, in agreement with others (5, 6), that there is some, but not a complete, inactivation of desoxycorticosterone acetate by the liver. The weight changes in the pellets used here were too small to permit a definite statement that the

absorption of hormone at the different sites of pellet implantation was at equal rates, but that it was probably equal is inferred from the analogous measurements of Burrill and Greene who used larger pellets.

The fact that desoxycorticosterone acetate given orally was less effective than that given parenterally correlates well with the idea that there is a partial destruction of the material by the liver. Grollman's conclusions (7) that the most effective feeding method is to mix the hormone with the dry food to obtain a relative constancy of intake was confirmed, but his conclusion that this is as effective a route of administration as subcutaneous injections was not.

No reason is apparent for the complete ineffectiveness of relatively large doses of desoxycorticosterone acetate when given in oil in single daily doses by stomach tube, but previous statements to that effect (2, 3) were fully confirmed.

It is to be emphasized that this loss of activity when desoxycorticosterone is absorbed from the gut or elsewhere through the portal system is not true of all cortical hormones. Natural secretions of transplants of the adrenal cortex were fully effective when absorbed through the liver (1) and various workers have found that cortical extracts and certain crystalline derivatives thereof are fully potent when given orally (2, 12). We obtained similar results with crystalline materials in experiments concerned with the maintenance of lactation in adrenalectomized rats (13).

Shimkin and White (14) have shown that if steroid hormones are dissolved in molten cholesterol, pellets can be obtained which are absorbed slowly and, in the instances tested, showed prolonged activity, as compared to pellets of the pure steroid. Whether or not this is true of the pellets of cholesterol and desoxycorticosterone acetate used here, they were remarkably effective. A single pellet contained about 0.75 mg. of desoxycorticosterone acetate. When placed at one of the better implantation sites these maintained life and good growth for over 30 days in most animals. If all of the hormone had been used in that time the average daily dose would have been 0.025 mg. per day. The hormone was not all absorbed, however, as indicated by the continued effectiveness of the pellets beyond 30 days, if they were left that long, and by their ability upon removal at 30 days to maintain other test animals into which they were re-implanted. The absorption rate from these pellets probably averaged less than 0.02 mg. per day. At least 0.05 mg. by injection is needed to give similar results (table 1, and unpublished observations). Most of the animals bearing 3 subcutaneous pellets, from which the absorption probably is about 0.05 mg. per day, grew at a normal rate and distinctly better than those receiving the same amount by injection.

In order for the rate of survival and growth to be roughly comparable to that reported by us, Burrill and Greene, who used somewhat older animals, used pellets from which 0.188 mg. daily was absorbed. Either the enhancing action of cholesterol in the pellets or dietary factors (7) may possibly have accounted in part for the greater effectiveness of the pellets used here, but the diet did not contain enough salt to sustain untreated animals.

SUMMARY

1. The effectiveness of desoxycorticosterone acetate in maintaining adrenalectomized rats is somewhat reduced when the substance passes through the hepatic portal system, probably indicating partial inactivation by the liver. This is true either for that absorbed from appropriately placed pellets, or for that given as a mixture in the dry food .No activity was observed when desoxycorticosterone acetate in oil was given in single daily doses by stomach tube.

2. Pellets were prepared from a mixture of cholesterol and desoxycorticosterone acetate, containing an average of 0.75 mg. of the latter. One such pellet at the better

implantation sites (subcutaneous, lymphatic, renal) maintained life and growth in most animals for more than 30 days, and 3 pellets maintained almost normal growth for similar periods.

REFERENCES

1. Eversole, W. J., A. Edelmann and R. Gaunt: *Anat. Rec.* 76: 271. 1940.
2. Kuizenga, M. H., J. W. Nelson and G. F. Cartland: *Am. J. Physiol.* 130: 1. 1940.
3. Spanhoff, R. W.: Acta brev. Neerland. 10: 92. 1940.
4. Eversole, W. J., and R. Gaunt: *Am. J. Physiol.* 133: 271. 1941.
5. Selye, H.: *J. Pharmacol. & Exper. Therap.* 71: 236. 1941.
6. Burrill, M. W., and R. R. Greene: 142. 1942.
7. Grollman, A.: *Endocrinology* 29: 862. 1941.
8. Mark, J., and G. R. Biskind: *Endocrinology* 26: 444. 1940.
9. Hartman, C. G.: *Endocrinology* 26: 449. 1940.
10. Ingle, D. J., and H. L. Mason: *Proc. Soc. Exper. Biol. & Med.* 39: 154. 1938.
11. Marrazzi, R., and R. Gaunt: *Proc. Soc. Exper. Biol. & Med.* 41: 65. 1939.
12. Grollman, A.: The Adrenals. Williams & Wilkins Co., Baltimore, 1936.
13. Gaunt, R., W. J. Eversole and E. C. Kendall: *Endocrinology* 26: 84. 1942.
14. Shimkin, M. B., and J. White: *Endocrinology* 29: 1020. 1941.
15. Forbes, T. R.: *Endocrinology* 30: 761. 1942.

Since this paper was submitted for publication Mark (*Endocrinology* 31: 582. 1942) reported he found no evidence that intrasplenic pellets of desoxycorticosterone acetate were less effective than those placed subcutaneously.

EFFECT OF PROPYLENE GLYCOL AND PROGESTERONE ON MOTILITY OF THE ISOLATED INTESTINE OF THE RABBIT

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THE REPORT of Dawson and Robson (1) strongly suggests that progesterone decreases the motility of the smooth muscle of the intestine. The problem is of practical importance as well as of theoretical interest. That progesterone does reduce the motility of the uterus is generally conceded and its inhibitory influence on the urinary bladder is confirmed by the work of Langworthy and Brach (2). Smith, Pomaranc and Ivy (3) observed 'only a trend toward an actual decreased reactivity of the gall bladder muscle itself' when experimenting with the gall bladder of pregnant animals and those injected with sex hormones.

Dawson and Robson (1) used absolute alcohol as a solvent for progesterone. Propylene glycol was used in these experiments as solvent since its toxicity has been found to be low (4). However, as no reports on the influence of propylene glycol on the motility of smooth muscle *in vitro* were available, it was deemed advisable to determine its effect under such circumstances. In a number of instances a fresh strip of gut was used for each determination as it has been intimated that tissue may develop a tolerance to certain drugs (5).

A strip of rabbit's intestine, about 3 cm. long, was suspended in 240 cc. of Sollmann-Rademaekers' solution and records of its activity made on a smoked drum, as is the custom with excised mammalian smooth muscle. The progesterone was dissolved in propylene glycol, 5 mg. per 1 cc. or 5 mg. per 2 cc. The threshold or adequate dose is used to indicate that concentration of the drug which produced an effect in the majority of instances.

EXPERIMENTAL RESULTS

Propylene glycol. In 25 experiments propylene glycol was used in concentrations of 1:1280 to 1:80 in Sollmann-Rademaekers' solution. The results showed some variation. Using 14 strips which had not been exposed to drugs previously, it was found that a concentration of 1:100 (2.4 cc. added to a bath of 240 cc.) or over usually stimulated the muscle. This was frequently manifested by an increased tonus but decreased amplitude of contraction and slight irregularities of rhythm. However, in some instances the amplitude of contraction was increased. Smaller doses may produce a slight and evanescent effect. In some instances even large doses produced only a temporary modification in motility, as the muscle recovered its normal rhythmicity in 15 to 25 minutes. After a response to propylene glycol if the muscle were washed and transferred to fresh solution it appeared to recover a practically normal rhythm in about 5 minutes. When a second dose of propylene glycol, equal to that of the first, was added to the bath the muscle responsed in a manner similar to the first response.

To confirm the above observations 11 strips were used each of which had been exposed to one or more of the following: epinephrine, progesterone and propylene glycol. The muscle had regained apparently an approximately normal rhythm. The

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results were substantially the same as indicated above. Moreover, in a number of the determinations, the amount of propylene glycol used was comparable to the amount used as solvent for a dose of progesterone. The variation in rhythmicity of the muscle was not significant when such a dose was used. There appeared to be little danger that the solvent of the progesterone would lead to a misinterpretation of results.

Progesterone. In 23 determinations progesterone was used in the bath in concentrations of 1:512,000 to 1:153,000. In 15 instances the drug was added to the bath in which was suspended a fresh segment of muscle. The tonus and amplitude of contraction were lessened in the majority of cases with a concentration of 1:256,000. This appeared to be the adequate or threshold dose. When 8 additional preparations were used which had been treated with propylene glycol or progesterone but which had resumed a suitable rhythmicity, the results were found to be similar to the above observation. The decrease in activity of the muscle was of a gradual nature and was exhibited over a considerable period of time.

As certain implications regarding the site of action of a drug on the cell have been deducted from the washout time and the effect of a second dose of the drug (5), it was deemed advisable to expose the muscle to a concentration of 1:256,000 of progesterone, record the result, remove and quickly wash the muscle, and record the effect of a second dose of the drug. Using 4 different preparations it was found that there was some difficulty in getting the rhythmicity back to normal after the first application of the drug. The average time of recovery to an approximate normal was about 14 minutes. A period of 3 minutes after the application of the drug was selected as an arbitrary point for determining the decrease in tonus of the muscle. After the first application the average decrease was about 18 mm., while after the second application it was about 13 mm. The mechanical advantage of the level was 3.5:19.5. Accordingly, the second application of the drug was hardly as effective as the first.

After 1 or more doses of progesterone had been applied to a strip, epinephrine chloride (0.12 to 0.03 cc. 1:1000 solution) was added to the bath of 240 cc. In 17 experiments an additional decrease in tonus was noted in each instance.

CONCLUSIONS

1. Propylene glycol in 1 per cent concentration generally modifies the motility of the intestine of the rabbit *in vitro*. The drug in this concentration has a stimulating effect on the muscle. The variations in motility caused by lesser concentrations of the drug are slight and evanescent. When placed in fresh Sollman-Rademaeker solution the muscle recovers rapidly and responds to a second dose in a manner similar to the first response.

2. Progesterone, in concentration of 1:256,000 and in greater concentration, depresses the motility of rabbit's intestine *in vitro*. Both tonus and amplitude of contraction are usually decreased. On placing the muscle in fresh Sollmann-Rademaeker's solution it regains an approximately normal rhythm in about 14 minutes. A second application of an adequate dose of the drug was found to be slightly less effective than the first. Epinephrine further depresses the intestinal muscle after progesterone.

I desire to express my appreciation to Doctor M. M. Cantor of the Department of Biochemistry for making available the progesterone.

REFERENCES

1. Dawson, R. F., and J. M. Robson: *J. Physiol.* 95: 420. 1939.
2. Langworthy, O. R., and C. B. Brack: *Am. J. Obst. & Gynec.* 37: 121. 1939.
3. Smith, J. J., M. M. Pomeranc and A. C. Ivy: *Am. J. Physiol.* 132: 129. 1941.
4. Seidenfeld, M. A., and P. J. Hanzlik: *J. Pharmacol. & Exper. Therap.* 44: 109. 1932.
5. Clark, A. J.: Mode of Action of Drugs on Cells. Edward Arnold & Co., London, 1933. p. 195.

EFFECT OF PREGNANCY AND LACTATION UPON THE THYROTROPIC HORMONE OF THE RABBIT[1,2]

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IT HAS BEEN SHOWN that during pregnancy the mammary glands develop under the influence of the hormones of the ovary and pituitary (1). Following parturition there is gradually initiated an intense secretion of milk which is continued for a variable period of time, determined in part by the persistency of the animal and in part by the nervous stimulation of regular milk removal. For a number of years this laboratory has been engaged in a study of the hormonal mechanisms involved in the growth of the mammary gland during pregnancy, and the initiation and maintenance of lactation post partum. One phase of this work has included a study of the concentration of certain hormones in the anterior pituitary gland during pregnancy and lactation in order to determine, if possible, the relation of the pituitary hormones to growth of the mammary gland and the lactation process.

As might be expected, the concentration of the lactogenic hormone of the anterior pituitary was found to change relatively little during pregnancy in the mouse (2), rat (3) and guinea pig (4) but was observed to reach a peak about 48 hours post partum, which was 2 to 3 times as high as that during pregnancy. In the rabbit (5) the lactogen content of the pituitary increased at least four-fold within 5 days after parturition.

The changes in the thyrotropin content of the pituitary during pregnancy and lactation are of especial interest because it has been shown that thyroidectomy decreases lactation and that even in normal animals the administration of thyroxine stimulates an increase in milk yield (6). The pituitaries of pregnant and lactating cattle (3) and rats (7) have been assayed for thyrotropic hormone. Here again the changes in the content of this hormone in the anterior pituitary during pregnancy have not followed a regular pattern but after parturition there was considerable evidence for a moderate increase during various stages of lactation.

In a previous paper, the change in concentration of the thyrotropic hormones in the anterior pituitary of the rabbit during growth has been reported (8). The object of the present paper is to record the results of a continuation of this work during the period of pregnancy and lactation. In addition, some slight evidence for the presence of the adrenotropic hormone is presented.

EXPERIMENTAL PROCEDURE

Normal New Zealand White female rabbits were used exclusively in this study. The method of feeding and care of the rabbits has been reported (8). Animals ranging in weight from 3000 to 4000 gm. were bred and then killed at definite periods of preg-

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nancy and lactation, as indicated in table 1. The pituitary, thyroids, adrenals and gonads were removed and weighed to the nearest 0.1 mg. on a precision balance. The pituitaries were frozen immediately and held in that condition until ready for assay.

As described in detail in previous papers (8, 9), one-day-old White Leghorn cockerels were used for the assay of the thyrotropic and the gonadotropic hormone. In addition, the weights of the adrenals of the assay chicks were recorded. The observations on the effect of pregnancy and lactation on the content of the gonadotropic hormone in the pituitary will be reported later. To obtain satisfactory adrenotropic hormone assays, larger amounts of anterior pituitary tissue will be required.

TABLE 1. THYROTROPIC HORMONE CONTENT OF THE PITUITARY OF PREGNANT AND LACTATING RABBITS

No. of Rabbits	Condition	Rabbit Body Wt., Av.	Rabbit Pit. Wt. Av.	Amount Pit. Injected per Chick	No. of Chicks in Group	Wt. of Chicks Av.	Chick Thyroid Wt., Av.	Chick Unit Content			Adrenal Increase of Injected Chicks	Thyroid Wt. in Rabbits, Av.	Adrenal Wt. in Rabbits, Av.
								per Gland	per Gram of pit.	per 100 gm. body wt.			
		gm.	mg.	mg.		mg.	mg.				%	mg.	mg.
10	Normal Controls	3054	31.2	13	20	54.5	5.12	1.981	63.46	0.065	20.52	164.2	281.5
8	Normal Controls	3544	45.1	14	20	53.5	5.12	2.655	58.93	0.075	6.57	167.3	399.9
8	Normal Controls	4021	41.4	15	20	53.8	4.91	2.020	48.80	0.050	13.32	149.2	531.7
6	Pregnant 10 days	3328	35.8	15	14	59.7	5.32	2.197	60.93	0.066	9.85	154.8	396.1
11	Pregnant 20 days	3701	36.7	15	20	54.1	5.42	2.341	63.87	0.064	14.77	152.7	480.2
7	Pregnant 28 days	3739	39.0	15	17	55.2	4.85	1.838	47.07	0.049	13.24	168.8	469.8
9	2 days post partum, young removed 15 hr.	3487	40.2	15	20	54.0	4.96	2.020	50.27	0.058	10.29	184.9	435.0
8	2 days post partum, young removed 12 hr. then allowed 3 hr. nursing	3638	40.0	15	18	54.9	4.98	2.033	50.87	0.056	8.42	174.8	431.6
7	5 days post partum, young removed 15 hr.	3327	46.0	15	11	53.5	5.05	2.434	52.93	0.075	16.08	163.3	349.9
7	5 days post partum, young removed 12 hr. then allowed 3 hr. nursing	3413	46.2	15	15	52.1	5.09	2.499	54.13	0.073	21.99	178.6	422.7
7	10 days post partum	3561	54.2	15	16	53.7	5.24	3.170	58.53	0.093	17.51	156.9	423.9
11	20 days post partum	3357	42.3	15	20	53.9	5.02	2.200	52.07	0.066	no increase	171.9	342.5
8	20 days post partum	3046	41.3	15	15	54.6	4.96	2.076	50.27	0.068	no increase	162.8	603.1
15	30 days post partum	3270	41.4	15	20	53.0	5.34	2.545	61.51	0.770		172.9	342.5

Assay of the pituitaries of the original group of rabbits which had been lactating 20 days, appeared to show a rather low concentration of the thyrotropic hormone. As a result pituitaries from a second group of animals were collected and the assay repeated with the results shown (table 1).

The technic earlier used to show the influence of the nursing stimulus upon the lactogenic hormone was repeated with the group of animals lactating for 2 and 5 days (3).

OBSERVATIONS

As the experimental animals varied in body weight from 3000 to 4000 gm., it seemed best to determine the influence of pregnancy upon the thyrotropic hormone in comparison with 3 groups of normal animals in this weight range, as previously presented (8). On the basis of the content of thyrotropic hormone *a*) per gland, *b*) per gram of pituitary and *c*) per 100 gm. of body weight, the pituitaries of pregnant animals yielded values within the range of the corresponding values for the non-pregnant normal females. Following parturition, there appears to be a tendency for the pituitary to become slightly heavier during the first 10 days of lactation; as a result there is a slight increase in the content of thyrotropic hormone per pituitary, without a corresponding increase in the concentration per gram of tissue. It thus appears that the

thyrotropic hormone of the rabbits under consideration did not vary sufficiently from the normal to indicate that either pregnancy or lactation influenced this factor. It is true that the pituitaries of the group which had been lactating 10 days contained the largest amount of thyrotropic hormone per pituitary of any group so far assayed.

This may be of considerable significance in the light of the fact that in normal growing female rabbits, the maximum thyrotropic hormone concentration in the anterior pituitary reaches a peak when the animal weighs about 2500 gm. The normal trend of the heavier animals included in this study would be downward, yet at the height of lactation (10 days) the thyrotropic hormone per anterior pituitary gland was maximal.

It is recognized that the weight of the thyroid gland of mature animals is not a satisfactory index of thyroid activity, due to the difficulty of interpreting the effect of colloid storage and discharge upon the weight of the gland. It is interesting to note, however, that the average thyroid weight for 26 normal rabbits weighing from 3000 to 4000 gm. was 160.5 mg., while that for 24 pregnant rabbits was 157.9 mg. The 72 lactating rabbits had thyroids weighing slightly more, 171.0 mg. It thus appears from these data that pregnancy had no effect, and lactation only a slight effect, on the thyroid weight.

It was shown further that neither the removal of the young for a period of 15 hours from the mothers, nor a period of nursing just before killing them influenced the concentration of the thyrotropic hormone in the pituitary. The two groups of animals, killed after 2 and 5 days of lactation respectively, are remarkably uniform in indicating a lack of effect on the thyrotropic hormone by the nursing stimulus which so markedly influences the lactogenic hormone.

The adrenal glands of the rabbits show a slight tendency to weight increase in late pregnancy and early lactation. The increase in the adrenal weight of the chicks receiving the rabbit pituitary was hardly sufficient to indicate greater adrenotropic activity at this time. However, there is evidence that the adrenals of the animals in intense lactation show the results of maximal adrenotropic activity. Inasmuch as all of the assays for the adrenotropic hormone were below the unit response, larger amounts of pituitary tissue will be required to indicate definitely whether reproduction and lactation influence the content of adrenotropic hormone.

DISCUSSION

Thyroxine, the hormone of the thyroid gland, has been recognized as a metabolic stimulant of somatic cells for many years. In the presence of increased amounts of thyroxine, the metabolism of the cells increases, resulting in an increased production of CO_2 and heat. Thus, one system of body heat regulation would operate as a result of the regulation of thyroxine secretion. It was not surprising to observe, therefore, upon the discovery of the relation of pituitary thyrotropin to thyroid activity, that low environmental temperature increased the production of thyrotropin and the cytological appearance of greater cellular activity in the thyroid and of colloid resorption. These observations explain the earlier observation of colloid storage with increased thyroxine potency in the thyroids of domestic animals during the summer months, and the gradual disappearance of colloid and increased cellular activity in the fall and winter months.

Since thyroid activity appears essential for the fulfilment of the potentialities for body growth, as is shown by the cessation of growth in the absence of the thyroid, it was not surprising to observe a rise in thyrotropin content of the anterior pituitary during the period of rapid growth and a decline during senescence (8). The mechanism

of this control is not so obvious. Is it possible that the inherent capacity of the cells of the young organism to function at a high level of metabolism in some way incites the thyrotropin-secreting mechanism of the anterior pituitary.

Lactation is a period of great metabolic activity. Large amounts of milk precursors must be digested, absorbed and mobilized constantly for the steady secretion of milk. The cells of the mammary glands are stimulated to secretory activity by the lactogenic hormone, yet the yield of milk appears to be influenced by the amount of thyroxine available. In the absence of the thyroid, lactation is considerably reduced but not stopped, whereas additional thyroxine in dairy animals increases and sustains the lactation curve.

On the basis of the observations in the rat (7) and cattle (3) it appeared that a mechanism similar to that which incited an increase in the lactogenic hormone in the anterior pituitary post partum might stimulate an increase in the rate of secretion of thyrotropin during lactation as well. In the absence of such an excitant it might be possible that increased thyroxine secretion could be stimulated as a result of the increased activity of the mammary gland. In other words, one type of thyroid control might originate in the demand of cells activated specifically by other hormones.

The data available on the thyrotropic content of the rabbit pituitary do not fit either theory suggested above. There is only a suggestion of increased thyrotropin secretion either during pregnancy or lactation. It is possible that factors as yet unrecognized and uncontrolled in our experiments, were sufficient to mask the influence of lactation upon the secretion of thyrotropin, or that small differences in the rate of thyrotropin secretion are sufficient to stimulate the increased secretion of thyroxine which might be required for lactation.

Possibly one reason why the injection of thyroxine or the feeding of thyroid substances stimulates increased and more prolonged lactation is that a mechanism is lacking in some species of mammals for inciting an increased secretion of thyrotropin and thyroxine following the initial stimulus to milk secretion afforded by the lactogenic hormone. Thus, there may be species differences in the necessity for additional thyrotropin to stimulate sufficient thyroxine to meet the requirements of lactation. It is well known that there are wide differences in the responsiveness of the thyroid of various species to given amounts of thyrotropin, even on the basis of equality in body weight. Similarly, there are great differences in the thyrotropin content of the anterior pituitary of various species. It has been suggested that there is an inverse relationship between thyrotropin concentration in the anterior pituitary gland and the sensitivity of the thyroid in the various species.

In the female rat, in which the thyrotropin content of the anterior pituitary gland is high and the sensitivity of the thyroid is low, there was observed an increase in thyrotropin during lactation. The anterior pituitary gland of the female rabbit contained only 1/10 as much thyrotropin per 100 gm. of body weight at a comparable stage of growth as the rat. Possibly the lowered concentration of thyrotropin in the rabbit anterior pituitary and the higher sensitivity of the thyroid to this hormone minimizes the change at the time of lactation and accounts for the species difference observed.

SUMMARY

Data are presented concerning the amount of thyrotropic hormone in the pituitary glands of female White New Zealand rabbits during pregnancy and lactation. The pituitaries were assayed by groups at 10, 20, and 28 days of pregnancy and at 2, 5, 10, 20, and 30 days post partum, using one-day-old White Leghorn male chicks. The results are expressed in terms of Bergman-Turner chick units. On the basis of the con-

tent of thyrotropin *a*) per gland, *b*) per gm. of pituitary and *c*) per 100 gm. of body weight, the values for the pregnant animals are within the range of the corresponding values for the non-pregnant normal females.

There was a tendency for the pituitaries to be slightly heavier during the first 10 days post partum. As a result there was a slight increase in the thyrotropin per anterior pituitary without a corresponding increase in the concentration per gram of tissue.

REFERENCES

1. Turner, C. W.: In Allen, E., ed. Sex and Internal Secretion. 2nd ed. *Williams & Wilkins, Baltimore*, 1939.
2. Hurst, V., and C. W. Turner: *Endocrinology*. 31: 334. 1942.
3. Reece, R. P., and C. W. Turner: *Mo. Agr. Exp. Sta. Res. Bull.* 266. 1939.
4. Holst, S., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 42: 479. 1939.
5. Meites, J., and C. W. Turner: *Endocrinology*. 31: 340. 1942.
6. Ralston, N. P., W. C. Cowsert, A. C. Ragsdale, H. A. Herman and C. W. Turner: *Mo. Agr. Exp. Sta. Res. Bull.* 317. 1940.
7. Turner, C. W., and P. T. Cupps: *Endocrinology* 24: 650. 1939.
8. Bergman, A. J., and C. W. Turner: *Endocrinology* 29: 313. 1941.
9. Bergman, A. J., and C. W. Turner: *Endocrinology* 24: 656. 1939.

METABOLISM OF ESTROGENS: EFFECT OF PREGNANCY UPON METABOLISM *IN VITRO* OF ESTRONE, ESTRADIOL AND ESTRIOL[1,2]

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WHEN KNOWN AMOUNTS of crystalline estrogens were incubated with tissue slices in a buffered medium and the mixture was subsequently assayed biologically (1), the following observations were made: *a*) α-estradiol was completely inactivated by liver tissue, partially inactivated by kidney and not at all by the other tissues tested. *b*) Estrone was completely inactivated by rabbit liver and only partially inactivated by rabbit kidney and rat liver. Estrone increased in biologic activity, however, after incubation with endometrium, ovary, spleen, heart and lung from the rat and rabbit. This increase in biologic potency of estrone by these tissues was ascribed to its conversion to α-estradiol by an enzymatic reduction system. The inactivation of estrone and α-estradiol by liver and kidney tissue was attributed to an oxidative enzyme system found in liver and kidney tissues only. When NaCN was used to inhibit the oxidative system in liver and kidney, and thereby prevented the destruction of α-estradiol, added estrone was shown to be converted to α-estradiol by kidney and liver also. It was concluded that estrone can be converted to α-estradiol by all of the tissues tested, and that its biologic activity is probably due to this conversion, estrone itself being inert. *c*) Estriol was only slightly inactivated by liver and kidney and therefore was relatively more effective when administered by the oral route.

It has been repeatedly suggested that pregnancy profoundly affects the metabolism of the estrogens through the presence of progesterone. Smith and Smith (2) first reported that progesterone added to administered estrogens caused an increased excretion of estrogens. Later Smith, Smith and Pincus (3) postulated that the presence of progesterone prevents the destruction of estrogens. Pincus and Zahl (4) and Pincus (5), using colorimetric determinations, also report that progesterone increases the amount of estrogens excreted and that progesterone (or active corpus luteum secretion) facilitates the conversion of estrone to estriol. These authors also postulate that in the presence of intact uterus and ovaries α-estradiol is transformed to estrone and estrone into estriol. These reactions do not take place in hysterectomized-ovariectomized rabbits. Fish and Dorfman (6) and Heard, Bauld and Hoffman (7),

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[2] We are indebted to Doctor G. Stragnell of the Schering Corp., Bloomfield, N. J., for the supply of estradiol and estrone, and to Doctor Oliver Kamm of Parke, Davis and Co., Detroit, Mich., for the estriol.

using chemical determinations, however, clearly showed that ovaries and uterus are not necessary for the conversion of α-estradiol to estrone and β-estradiol. A complete review of the subject and excellent bibliography can be found by referring to Doisy, Thayer and Van Bruggen (8).

To investigate further the role of progesterone in the metabolism of the crystalline estrogens, α-estradiol, estrone and estriol, comparative studies by incubation were made using pregnant and non-pregnant rats and rabbits as the source of the tissues. Slices of tissue weighing 250 mg. from liver, kidney, spleen and heart, and a similar quantity of minced uterus were used. The technical procedures were identical to those of the previous study (1). Estrogens were assayed by the increase in weight of the albino rat uterus (9).

A study of table 1 reveals that tissues from pregnant and non-pregnant animals

TABLE 1. COMPARISON OF EFFECT OF TISSUES FROM PREGNANT AND NON-PREGNANT ANIMALS ON THE METABOLISM OF α-ESTRADIOL

Tissue	Theoretical[1] Amount of α-Estradiol Injected in mg.	Assay by Uterine Response, Average Weight in mg.			Number of Assay Rats		
		Controls	Incubation with Animal Tissues		Controls	Non-Pregnant	Pregnant
			Non-Pregnant	Pregnant			
None	None	19.7			85		
None	0.3	89.3			25		
	0.9	90.0			15		
Liver, rabbit	0.3		23.3	22.6		9	3
	0.9		40.8	46.0		5	2
Liver, rat	0.3		20.6	22.8		29	5
	0.9		19.9	23.3		3	3
Kidney, rabbit	0.3		27.0	24.0		6	5
	0.9		48.0	38.0		6	2
Kidney, rat	0.3		73.1	65.6		25	5
	0.9		90.0	122.0		1	2
Uterus, rabbit	0.3		76.6	87.4		6	5
Uterus, rat	0.3		78.7	92.1		9	6
Heart, rat	0.3		82.0	92.0		1	1

[1] Amount of α-Estradiol added to flasks before incubation with tissues.

of the same species have a similar effect on the metabolism of α-estradiol. Liver from pregnant and non-pregnant rats completely inactivates α-estradiol in amounts up to 0.9 mg. A threshold for inactivation by the liver was reached at the 0.9 mg. level for rabbit liver and no measurable difference was noted between liver from pregnant and non-pregnant animals. A threshold for inactivation by the kidney tissue was obtained with both rat and rabbit kidney and again no significant difference was found in those derived from pregnant and non-pregnant animals. Addition of α-estradiol to heart slices or uterine mince in no case altered the biologic activity of the added estrogen.

Estrone (table 2), was likewise metabolized identically, whether tissues came from pregnant or non-pregnant animals. One exception is noted, that tissue from the non-pregnant rat uterus was less effective in increasing the potency of estrone than that from pregnant rat uterus. This was later shown to be due to the difference in proportion of endometrium to muscle in the tissue slice. Hog uterus is also capable of increasing the biologic activity of estrone. This is true, to the same degree, of both pregnant and non-pregnant hog uterus.

XUM

Estriol too (table 3), was metabolized in the same manner by tissues from pregnant as well as from non-pregnant animals.

We were therefore forced to conclude, after using 3 different estrogens, a range of doses, 3 species of animals, and a wide variety of tissues, that pregnancy does not alter the metabolism of estrogens *in vitro*. When this is considered along with the similar chemical findings *in vivo* of Heard (7), one doubts that a true qualitative or quantitative difference exists in pregnancy. Thus, both biologic and chemical determinations cast doubt on the colorimetric findings reported (3, 4, 5.) Although the

TABLE 2. COMPARISON OF EFFECT OF TISSUES FROM PREGNANT AND NON-PREGNANT ANIMALS ON THE METABOLISM OF ESTRONE

Tissue	Theoretical[1] Amount of Estrone Injected in mg.	Assay by Uterine Response, Average Weight in mg.			Number of Assay Rats		
		Controls	Incubation with Animal Tissues		Controls	Non-pregnant	Pregnant
			Non-pregnant	Pregnant			
None	None	19.7			85		
None	0.3	19.7			85		
	1.0	38.5			34		
	2.0	55.6			28		
Liver, rabbit	0.3		19.0	23.3		4	3
	0.9		22.2	23.0		5	2
	1.8		26.0	32.0		3	2
Liver, rat	0.9		39.5	25.0		4	6
Kidney, rabbit	0.3		20.0	23.6		2	5
	0.9		40.0	35.0		2	2
	1.8		62.5	59.0		2	2
Kidney, rat	0.3		56.0	40.0		3	3
	0.9		81.0	87.2		4	4
Spleen, rabbit	0.9		56.0	57.0		1	1
Spleen, rat	0.3		34.8	30.0		5	2
Uterus, rabbit	0.3		83.1	72.6		10	10
	0.9		101.5	97.7		7	4
Uterus, rat	0.3		28.3[2]	76.5		8	6
Uterus, hog	0.3		61.6	61.2		9	12
	0.6		78.4	84.3		7	9

[1] Amount of estrone added to flasks before incubation with tissues.
[2] Lack of conversion due to smaller amount of endometrial tissue.

uterus converts estrone to α-estradiol, no evidence of conversion of estrone to estriol, or of estradiol to estrone was encountered.

Doisy, et al. (8), on the basis of urinary excretion findings, believe that in the monkey the β-estradiol administered is transformed thus:

(β-estradiol)→estrone→α-estradiol

While in the rabbit, Fish and Dorfman (6) and Heard, et al. (7) have shown that the α-estradiol administered takes the opposite path of transformation:

α-estradiol→estrone→(β-estradiol)

Our studies *in vitro* indicate that possibly these reactions are reversible under different conditions since the reaction is:

estrone→α-estradiol→oxidative destruction

β-estradiol was not added to the system nor tested for. All of the added estrone, how-

TABLE 3. COMPARISON OF EFFECT OF TISSUES FROM PREGNANT AND NON-PREGNANT ANIMALS ON THE METABOLISM OF ESTRIOL

Tissue	Theoretical[1] Amount of Estriol Injected in mg.	Assay by Uterine Response, Average Weight in mg.			Number of Assay Rats		
		Controls	Incubation with Animal Tissues from		Controls	Non-pregnant	Pregnant
			Non-pregnant	Pregnant			
None	None	19.7			85		
None	0.3	35.0			10		
	0.5	47.0			3		
	1.0	53.0			4		
Liver, rabbit	0.3		26.5	30.0		7	5
	0.9		44.3	30.5		6	2
Liver, rat	0.3		26.0	25.5		5	6
	0.6		31.1	35.6		6	5
Kidney, rabbit	0.3		39.0	42.0		6	6
	0.9		58.0	54.0		3	2
Kidney, rat	0.3		27.8	20.2		18	4
Uterus, rabbit	0.3		38.7	42.7		4	4
	0.9		54.5	53.0		2	1
Uterus, rat	0.3		40.0	42.0		3	2

[1] Amount of estriol added to flasks before incubation with tissues.

ever, can be accounted for as α-estradiol in both cyanide-poisoned liver and kidney and in endometrium. That such a transformation should be reversible under different conditions seems logical since the conversions are enzymatic in nature (1), and most enzymatic processes are reversible reactions. All of the above-mentioned transforma-

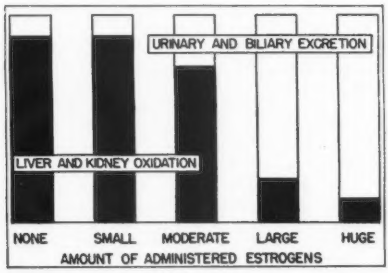

Fig. 1. RELATIVE RÔLES PLAYED BY URINARY EXCRETION, BILIARY EXCRETION AND OXIDATION by liver and kidney in the metabolism of estrogens under varying circumstances.

tions take place at the 17-position. According to Segaloff (21) the destruction takes place at the 17-position also.

What is the physiologically normal route involved in the metabolism of the estrogens? Since Zondek (10) has shown that only negligible amounts of estrogens are stored by the body or eliminated in the intestines, these routes can be disregarded. Dingemanse and Laqueur (11) report that 90 per cent of added estrogen is destroyed by the body. This destruction takes place mainly in the liver (1, 12, 13, 14, 16, 17). From 2 to 20 per cent of administered estrogens can be recovered in the urine. This mechanism has been widely studied. Estrogens are also excreted in the bile (18, 19). The latter authors found no estrogens in the bile of untreated dogs, but recovered

40 to 48 per cent of a huge intravenous dose of estrone (250,000 I.U.) from the common duct. Their conclusion that estrogens are not rapidly destroyed or inactivated by the liver is open to question because of *a*) the unphysiological doses, *b*) the fact that the recovered estrogen may have been the more potent α-estradiol and *c*) by their failure to find estrogens in the bile of normal dogs.

Figure 1 schematically illustrates the relative roles played by *a*) urinary and biliary excretion and *b*) oxidation by liver and kidney in the metabolism of α-estradiol and estrone. The liver and kidneys have a definite threshold capacity for oxidizing α-estradiol. Any amount above the threshold will escape oxidation. When an intact animal is given more estrogen than the liver can oxidize, the blood level will be elevated and greater biliary excretion and urinary excretion are possible. At least two mechanisms for dealing with estrogens occur in the body, *a*) an oxidative mechanism which inactivates the greater part of physiologically circulating estrogens, and *b*) an overflow mechanism which operates mainly after liver oxidative capacity is reached, or when the liver is damaged. The latter has been well illustrated by Talbot (14), Pincus and Martin (20), and Biskind (16).

The main metabolic routes for inactivation of estrogens are probably not conjugation of free estrogens or transformations of one estrogen into another. These routes are involved mainly in the urinary excretion of estrogens, which constitutes only 2 and at most 20 per cent of the total estrogen inactivation. They are also used when the liver oxidative system has been saturated.

These postulations apply only to the 3 naturally occurring estrogens studied.

We wish to express appreciation to Doctor Gordon B. Myers, Professor of Medicine, Wayne University College of Medicine, for his assistance in the preparation of this paper.

REFERENCES

1. Heller, C. G.: *Endocrinology* 26: 619. 1940.
2. Smith, G. V. S., and O. W. Smith: *Am. J. Physiol.* 98: 578. 1931.
3. Smith, G. V. S., O. W. Smith and G. Pincus: *Am. J. Physiol.* 121: 98. 1938.
4. Pincus, G., and P. A. Zahl: *J. Gen. Physiol.* 20: 879. 1937.
5. Pincus, G.: *Cold Spring Harbor Symposium on Quantitative Biology* 5: 44. 1937.
6. Fish, W. R., and R. I. Dorfman: *J. Biol. Chem.* 140: XL. 1941.
7. Heard, R. D. H., W. G. Bauld and M. M. Hoffman: *J. Biol. Chem* 141: 709. 1941..
8. Doisy, E. A., S. A. Thayer and J. T. Van Bruggen: Federation Proceedings 1: 202. 1942.
9. Lauson, H. D., C. G. Heller, J. B. Golden and E. L. Sevringhaus: *Endocrinology* 24: 35. 1939.
10. Zondek, B.: Clinical and Experimental Investigations on the Genital Functions and Their Hormonal Regulation. Williams & Wilkins, Baltimore, 1941.
11. Dingemanse, E., and E. Laqueur: *Am. J. Obst. & Gynec.* 33: 1000. 1937.
12. Golden, J. B., and E. L. Sevringhaus: *Proc. Soc. Exper. Biol. & Med.* 39: 361. 1938.
13. Zondek, B.: *Skandinav. Arch. f. Physiol.* 70: 133. 1934.
14. Talbot, N. B.: *Endocrinology* 25: 601. 1939.
15. Biskind, G. R., and J. Mark: *Bull. Johns Hopkins Hosp.* 65: 212. 1939.
16. Biskind, M. S., and G. R. Biskind: *Endocrinology* 31: 109. 1942.
17. Segaloff, A., and W. O. Nelson: *Proc. Soc. Exper. Biol. & Med.* 44: 39. 1941.
18. Longwell, B. B., and F. S. McKee: *J. Biol. Chem.* 142: 757. 1942.
19. Cantarow, A., A. E. Rakoff, K. E. Pashkis and L. P. Hansen: *Endocrinology* 30: S1027. 1942.
20. Pincus, G., and D. W. Martin: *Endocrinology* 27: 838. 1940.
21. Segaloff, A.: Unpublished data.

EFFECTS OF SEX HORMONES UPON BODY GROWTH, SKIN, HAIR AND SEBACEOUS GLANDS IN THE RAT[1]

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FROM THE BEGINNINGS of endocrinology it has been generally believed that the sex hormones are somehow involved in the control of the growth of hair and of the texture of the skin. It is only recently, however, that the problem has begun to attract the serious attention of endocrinologists. In experimental animals administration of large amounts of estrogen for sufficient periods of time has been followed by a failure of regrowth of hair in rats (1, 2, 3) dogs (4) and guinea-pigs (5). In man Hamilton (6, 7) found that administration of androgens resulted in greater growth of body hair, and at the same time occasionally precipitated the onset of baldness. Hamilton (8) has also presented evidence that androgen is an incitant of acne and that this substance apparently stimulates secretory activity in human sebaceous glands.

The observations described here were made in the course of experiments which were conducted in an attempt to analyze the impaired growth of estrogen-treated rats (1, 2, 9, 10, 11). In other experiments it had been repeatedly observed that administration of estrogen to rats inhibited both somatic growth and the growth of hair. These two effects, and the well-known cornification of the vagina and cervix and of the male accessory glands of reproduction in estrogen-treated rats, may possibly involve changes in cystine metabolism as a factor common to all three phenomena. It might be assumed, for example, that genital cornification under the stimulus of estrogen might deplete the stores of cystine needed for growth of hair and for body growth. On the other hand, it has been reported (12) that estrogen may be excreted as a sulfur compound, and it might be assumed that such inactivation of the excessive estrogen would result in a sulphur or cystine deficiency. Increasing the dietary intake of cystine or methionine has ameliorated the stunting of rats under treatment with carcinogenic chemicals (13), apparently by relieving a cystine deficiency resulting from the detoxification of the carcinogen. Cystine has also been reported to stimulate growth of hair in genetically hypotrichotic rats (14). The present experiments, therefore, consisted of examination of the effects of increased cystine intake and of administration of androgen upon somatic growth and hair growth in estrogen-treated rats.

MATERIALS AND METHODS

A total of 69 rats ranging in weight from 40 to 145 gm. at the start of the experiment were used. The basic diet in all cases was a commercial preparation, Tioga dog

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[1] This study was aided by grants from the Fluid Research Fund of Yale University School of Medicine.

food.[2] The hormone substances were administered twice weekly by subcutaneous injection. The estrogen[3] chosen was estradiol benzoate and the dose in all cases was 83 micrograms. The androgen was testosterone propionate,[3] and here the dose was 2.5 mg. These substances were in solution in sesame oil in such concentration that a dose was contained in 0.05 and 0.1 cc., respectively, of the oil.

In one experiment 23 rats were placed on a diet of finely ground Tioga dog food. Of these animals 6 (3 males, 3 females) received no treatment, 8 (2 males, 6 females) were given estrogen, and 9 (3 males, 6 females) were given estrogen and the diet was supplemented with 20 parts of casein as a source of cystine. A second experiment employed 14 rats. Ten of these animals (6 males, 4 females) were treated with estrogen

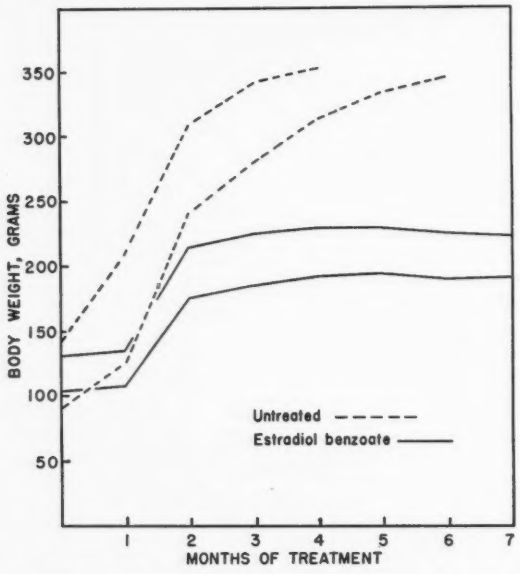


Fig. 1. Growth of estrogen-treated rats compared with untreated rats. Each curve represents a single animal.

and 4 (2 males, 2 females) were kept untreated. After 3 months all of these animals were placed on a diet of finely ground food, and one month later 400 mg. of l-cystine to each 100 gm. of ground food was added to the diet of 6 of the estrogen-treated animals (3 males, 3 females). A final experiment employed 32 rats, all of which were fed ground food from the start. Four of these animals were kept untreated and 28 were given estrogen. In addition to the estrogen, 6 (3 males, 3 females) were given androgen and 10 males were given the dietary supplement of l-cystine (400 mg. in each 100 gm. of ground food), leaving 12 animals (10 males, 2 females) receiving only estrogen and the basic diet.

This regime was maintained for 6 to 10 months. The animals were weighed at weekly intervals, and the condition of the hair and skin was noted. At the end of the experiment the animals were killed with ether and, in the case of the last 52 animals,

[2] Obtained from Tioga Mills, Inc., Waverly, N. Y.

[3] The estradiol benzoate and testosterone propionate were supplied by the Shering Corporation through the courtesy of Dr. E. Schwenk.

weights were recorded for the testes, ovaries, accessory glands of reproduction, adrenal glands, pituitary bodies and kidneys. In the last experiment a strip of skin was removed at autopsy from the back, belly and right thigh of all of the animals and preserved. Bouin's fluid was used as a fixative and, after embedding in paraffin, sections were cut at 10 micra and stained with hematoxylin and eosin.

OBSERVATIONS

Body growth. The 24 animals on the basic diet and receiving estrogen showed a distinct inhibition of growth (fig. 1). In 7 animals the inhibition became apparent immediately and persisted. In 10 animals the stunting set in during the second month and in 3 animals during the third month. In 4 animals growth ceased during the first month of treatment, recovered during the second month, and stunting became persistent during the third month (fig. 1, 2, 3). The degree of inhibition was essentially the same in both sexes.

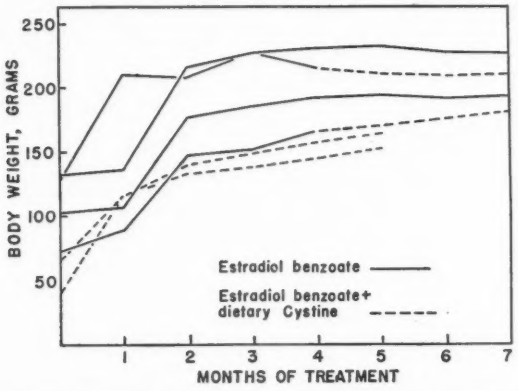


Fig. 2. GROWTH OF ESTROGEN-TREATED RATS WITH AND WITHOUT THE ADDED DIETARY CYSTINE. Each curve represents a single animal to show the variation in the onset of persistent stunting.

Supplementing the basic diet with cystine as such or as casein had no consistent influence in ameliorating the inhibition of growth (fig. 2). In an occasional animal given the supplement, growth was less inhibited than in the average of the estrogen-treated animals not given added cystine; but this difference did not exceed the range of variation in the latter group of animals. From time to time the daily consumption of food was determined in all groups and was found to be essentially the same, indicating that the effects upon growth are probably not to be attributed to differences in food consumption.

There was variation in the growth of the animals on the basic diet receiving testosterone propionate in addition to the estrogen (fig. 3). In the 3 females the androgen had no effect upon the extent of the inhibition of growth by the estrogen, while the 3 males receiving both hormone substances grew at a rate intermediate between that of the untreated animals and of the animals receiving estrogen alone. Here again, the amount of food consumed daily did not appear to be the responsible factor.

Organ weights. As shown in table 1, the weighed organs exhibited the changes usually reported for rats treated with estrogens or estrogens and androgens. The gonads and the male accessory glands of reproduction were substantially damaged by the administration of estrogen, while the androgen conferred some degree of 'protection' against this effect of the estrogen. The adrenals were not consistently altered in

weight. The kidneys were reduced in size in the estrogen-treated animals and hypertrophied in the rats receiving both estrogen and androgen. The dietary supplement of cystine was without influence on the action of the estrogen upon the weights of these organs. Hypertrophy of the hypophysis occurred in all of the estrogen-treated

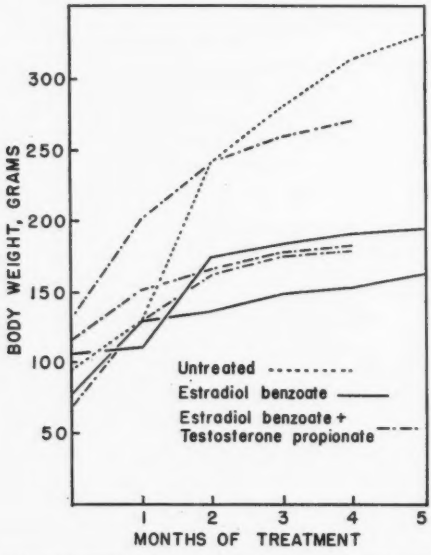


Fig. 3. GROWTH OF RATS RECEIVING ESTROGEN AND ESTROGEN AND ANDROGEN AS COMPARED WITH THAT OF AN UNTREATED ANIMAL. The two animals given androgen and showing marked suppression of growth were females; the animal similarly treated and showing less suppression was a male.

rats, the degree of hypertrophy apparently depending upon the duration of treatment. Pituitary hypertrophy was present also in the animals given the added dietary cystine; but in these animals the hypertrophy was significantly less than in the estrogen-treated animals not given added cystine. No significant pituitary hypertrophy was seen in the animals receiving both estrogen and androgen.

Hair growth. In the animals kept on the basic diet and given estrogen, the coats

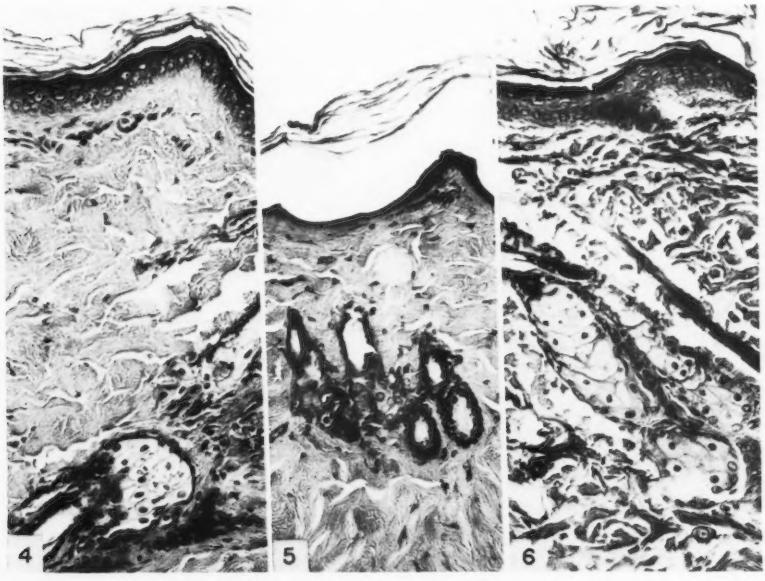
TABLE 1. WEIGHTS OF ORGANS OF RATS AFTER 4 TO 8 MONTHS' TREATMENT WITH ESTROGEN, ESTROGEN AND ADDED DIETARY CYSTINE, AND ESTROGEN AND ANDROGEN

Treatment	Sex	Number	Body Weight, gm.	Testes, gm.	Accessories,[1] gm.	Ovaries, mg.	Adrenals, mg.	Kidneys, gm.	Hypophysis, mg.
None	M	6	303(210–385)	2.53(1.7–3.0)	1.3(0.78–2.20)		36.6(26.0–48.0)	2.4(1.8–2.8)	8.2(5.0–15.0)
	F	3	239(192–265)			71.8(60.0–83.5)	62.3(55.5–78.0)	2.1(1.8–2.5)	13.0(12.0–15.0)
Estrogen	M	12	205(155–239)	0.38(0.2–0.62)	0.22(0.16–0.32)		52.7(39.5–61.0)	2.0(1.5–2.6)	47.1(22.0–218.0)
	F	7	166(150–188)			8.0	45.8(33.0–59.0)	1.7(1.1–2.1)	52.0(21.0–83.0)
Estrogen & Cystine	M	14	192(157–228)	0.30(0.15–0.50)	0.24(0.16–0.33)		54.1(42.0–67.5)	1.9(1.5–2.4)	32.2(20.0–59.5)
	F	7	182(156–222)			9.2(6.0–13.5)	46.6(37.0–58.0)	1.7(1.3–2.0)	34.9(22.0–44.0)
Estrogen & Androgen	M	3	264(249–273)	0.62(0.40–0.82)	2.28(2.05–2.45)			3.6(2.8–4.2)	12.6(12.0–14.0)

[1] Seminal vesicles, coagulating glands and prostate glands.

became shaggy with a thinning of the hair and the appearance of areas almost denuded of hair. This effect became evident after 2 months of treatment, progressed slowly, and was seen in all of the animals on this treatment. In a number of these animals several areas, such as belly, dorsum, flank and thigh were shaved after the shagginess had become evident. Regrowth of hair in the shaved areas required approximately 3 times as long as regrowth in similar shaved areas of untreated rats. The time relations were the same for the first appearance of hair as for the attainment of full growth to the length of adjacent hair. The alopecic areas showed no dermatosis.

The animals receiving testosterone propionate in addition to the estrogen ex-



VERTICAL SECTIONS OF THE SKIN OF RATS (×175). Note especially the comparative thickness of the epidermis, the size and character of the sebaceous glands. Fig. 4. Untreated male, 12 months of age. Fig. 5. A male after 10 months' administration of 83 μg. or estradiol benzoate twice weekly. Fig. 6. A male after 7 months' administration of 83 μg. of estradiol benzoate and 2.5 mg. of testosterone propionate twice weekly. The distortion of the dermis is an artifact.

hibited no thinning of the hair or shagginess. Their coats were in all evident respects like those of the untreated animals. Similarly, shaved areas regrew hair as fully and as rapidly in these animals as in the untreated animals.

The addition of cystine or casein to the diet of the estrogen-treated rats had little effect on the extent or rapidity of loss of hair. Regrowth of hair in shaved areas usually was also unaffected by addition of cystine or casein to the diet. In two such animals hair grew in shaved areas even more promptly than in the untreated animals; but this observation might have been the result of differences in the hair cycle (15) at the time of shaving. This observation could not be duplicated in these or the other animals receiving the supplemented diet.

Skin. After two months of administration of estrogen an effect upon the skin was evident. Upon grasping the skin for the injections, it was readily felt to be distinctly thinner and more loosely bound than in the animals receiving testosterone propionate

in addition to estrogen. Another difference was the markedly greater ease with which a needle pierced the skin of the estrogen-treated animals. In these respects the skin of the animals receiving simultaneously both testosterone propionate and estrogen was indistinguishable from that of the untreated animals. No differences were apparent in the skin of the estrogen-treated animals given dietary supplements of cystine and those not given this supplement.

Microscopically, it was found in the estrogen-treated animals that all of the layers of the skin were greatly thinned (fig. 4, 5). The epidermis was but one or two cells thick in contrast to the 5 or 6 layers of cells in the untreated animals. The dermis was, typically, not more than one half as thick as in the untreated animals. The tela subcutanea was likewise thin in the estrogen-treated animals. Adipose tissue in this layer was much reduced or absent; indeed, in many instances the dermis appeared to rest directly upon the superficial muscle layer. The dietary supplement of cystine had little effect upon the thinning of the layers of the skin, with the exception that in these animals the epidermis was perhaps slightly less severely thinned. The animals given testosterone propionate in addition to the estrogen were apparently 'protected' completely against the thinning effects of the estrogen upon the skin (fig. 6). Indeed, all of the layers of the skin were perhaps significantly thicker than in the untreated animals. Whether the thinner epidermis in the animals receiving estrogen and no androgen was due entirely to atrophy or partially to earlier keratinization could not be determined. No differences from group to group in cutaneous vascularity were apparent in the sections.

Probably the most striking cutaneous effects of the hormones were upon the sebaceous glands. These glands in the untreated rat (fig. 4) of both sexes are not particularly large, but one or more accompany each hair shaft and they have essentially the same structure as the human sebaceous gland. In the animals on the basic diet receiving the estrogen, the sebaceous glands were much reduced in number, less than one-third as many being present in sections of comparable size. In addition to being much less numerous, the individual sebaceous glands (fig. 5) were not more than one-fifth as large as in the untreated animals. There was considerable variation in both number and size of the sebaceous glands in the estrogen-treated animals, and the numbers and dimensions given represent the upper limit of the range. In many of these animals the sebaceous glands were nearly obliterated, and the small number to be found consisted of but 3 or 4 cells. In the animals receiving estrogen and the dietary supplement of cystine no marked 'protection' of the sebaceous glands was apparent, although these glands in all of the members of this group were comparable to the best preserved sebaceous glands of the estrogen-treated animals not given the supplement of cystine. In the animals receiving both estrogen and androgen the sebaceous glands showed none of the effects of estrogen. Instead, these glands were even more numerous and much larger (fig. 6) than in the untreated animals.

The hair follicles and shafts in the several groups exhibited the morphology which would be expected from the gross observations. In the animals receiving only estrogen, their structure was characterized by an atrophic appearance as compared with those of the untreated animals. The dietary supplement of cystine was entirely without evident influence. In the animals receiving both estrogen and androgen the hairs presented a vigorous, healthy appearance, and microscopic evidence of growth, such as mitoses, was readily apparent.

DISCUSSION

Generally speaking, the observations made on the sebaceous glands in the course of these experiments offer an interesting parallel to the recent reports on the effects

of sex hormones in acne. If it be assumed that acne is related to increased activity or hypertrophy of the sebaceous glands, the hypertrophy in the rats receiving simultaneously both estrogen and androgen is in line with the appearance of acne after administration of androgens to human beings (8). The atrophy of the sebaceous glands in the rats given estrogen alone similarly recalls the report (16) of improvement in acne upon treatment with estrogen. The studies on acne suggest an action of sex hormones upon sebaceous glands, and in the rat such an action is clearly shown. It should be emphasized, however, that this study throws no light upon the action of androgen when administered alone.

The observations as to the effects of estrogen and androgen upon the epidermis may prove to have some bearing upon experimental epidermal tumors. It has been found that application of estradiol benzoate accelerates both the induction of epidermal carcinomas and the transformation of benign epidermal tumors into malignant tumors in mice receiving methylcholanthrene (17). There is some evidence that a phase of atrophy or aplasia precedes neoplasia in the development of certain induced tumors (18, 19). If this sequence is of general application, the estrogen may, by producing epidermal atrophy and thus providing a favorable field, expedite the strictly carcinogenic action of the methylcholanthrene. In this event administration of androgen should negate the accelerating influence of the estrogen and should retard the appearance of epidermal tumors. This possibility could, of course, exist only if estrogen has the same influence upon the epidermis in mice as in rats.

The mode of action of the steroids in producing the effects upon the skin is not known. Histologic study and the gross observations revealed no differences from group to group in the vascularity of the skin although vascular changes in the skin upon administration of sex hormones are well known (20, 21). Whether the action upon the skin is associated with the androgenicity or estrogenicity of the compounds used or is an action peculiar to these specific compounds cannot, of course, be predicted without further study.

The partial or complete 'protection' conferred by the androgen against the several effects of estrogen administered alone is much the same general reaction as seen in the bones of mice (22) and in the comb of the fowl (23, 24). Whether this is antagonism in the classical sense is, of course, conjectural. In view of these apparently antagonistic actions of the sex hormones, qualitatively comparable effects of estrogens and androgens, as upon mammary growth (25), for example, are somewhat paradoxical.

Administered estrogen produces inhibition of growth presumably by suppressing the release or production of hypophysial growth hormone (2, 11, 26). Why there should have been such variation in the time of onset of persistent stunting in our animals is not clear. A more puzzling situation is the immediate stunting followed by recovery and then by persistent stunting which occurred in a number of the animals receiving injections of estrogen. The growth of the animals receiving estrogen and androgen concurrently resembled that reported by Deanesly and Parks (27). These authors found growth less inhibited in male rats receiving testosterone propionate and stilbestrol than when only stilbestrol was administered. They also found that implantation of testosterone propionate alone inhibited growth, and that this effect was greater in females than in males.

The failure of an increased dietary cystine intake to affect the inhibition of hair growth and body growth by estrogen suggests that these effects of the estrogen are not primarily the result of an effective depletion of stores of cystine needed for normal growth of hair and of the body. It is possible, of course, that the dietary supplement was inadequate, although the same amount of cystine completely protected rats

against the stunting induced by carcinogens (13). The failure of added cystine to prevent loss of hair in the estrogen-treated animals recalls the report (28) of failure to confirm the observation (14) that administration of cystine to genetically hypotrichotic rats results in growth of hair, although the two situations (genetic and estrogen-induced) probably have quite different backgrounds. The observation that hypophysial enlargement was significantly less in the estrogen-treated animals given added dietary cystine may assume importance in the analysis of the induction of pituitary tumors in rats by administration of estrogen.

SUMMARY

The subcutaneous injection of 83 micrograms of estradiol benzoate twice weekly was followed by a marked suppression in the growth of rats. Concurrent administration of 2.5 mg. of testosterone propionate twice weekly did not offset the stunting of growth in the females, but in males the stunting was much reduced. Supplementing the colony diet with l-cystine had no effect upon the growth-suppressing action of the estrogen.

Shagginess and marked loss of hair were present in all of the estrogen-treated animals, and regrowth of hair in shaved areas was greatly retarded. Although added dietary cystine did not modify the effect of the estrogen upon the hair, the animals receiving the androgen in addition to the estrogen were completely 'protected' against the effects of the estrogen upon the hair.

The skin of the estrogen-treated rats was quite thin, with both dermis and epidermis being so affected. The sebaceous glands were much reduced in size and number. Added dietary cystine did not significantly alter these actions of the estrogen. On the other hand, the animals receiving both the androgen and estrogen were completely 'protected' against these changes.

REFERENCES

1. Zondek, B.: *Lancet* 1: 10. 1936.
2. Zondek, B.: *Folia clin. Orientalia* 1: 1. 1937.
3. Forbes, T. R.: *Endocrinology* 30: 465. 1942.
4. Gardner, W. U., and J. De Vita: *Yale J. Biol. & Med.* 13: 213. 1940.
5. von Wattenwyl, H.: *Schweiz. med. Wchenschr.* 71: 1331. 1941.
6. Hamilton, J. B.: *Endocrinology* 21: 649. 1937.
7. Hamilton, J. B.: *Anat. Rec.* 82: 26 (Suppl). 1942.
8. Hamilton, J. B.: *J. Clinical Endocrinology* 1: 570. 1941.
9. Wade, N. J., and E. A. Doisy: *Proc. Soc. Exper. Biol. & Med.* 28: 714. 1931.
10. Spencer, J., F. E. D'Amour and R. G. Gustavson: *Am. J. Anat.* 50: 129. 1932.
11. Deanesly, R.: *J. of Endocrinology* 1: 36. 1939.
12. Schachter, B., and G. F. Marrian: *J. Biol Chem.* 126: 663. 1938.
13. White, J., and A. White: *J. Biol. Chem.* 131: 149. 1939.
14. Martin, G. J., and R. E. Gardner: *J. Biol. Chem.* 111: 193. 1935.
15. Butcher, E. O.: *Anat. Rec.* 61: 5. 1934.
16. Lawrence, C. H., and N. T. Werthessen: *Endocrinology* 30: S1034. 1942.
17. Paletta, F. X., and P. F. Max: *J. Nat. Cancer Inst.* 2: 577. 1942.
18. Gardner, W. U., and E. Allen: *Yale J. Biol. & Med.* 12: 213. 1939.
19. Hooker, C. W., and C. A. Pfeiffer: *Cancer Research.* 2: 759. 1942.
20. Reynolds, S. R. M., and F. I. Foster: *J. Pharmacol. & Exper. Therap.* 68: 173. 1940.
21. Edwards, E. A., J. B. Hamilton, S. G. Duntley and G. Hubert: *Endocrinology* 28: 119. 1941.
22. Gardner, W. U., and C. A. Pfeiffer: *Proc. Soc. Exper. Biol. & Med.* 38: 599. 1938.
23. Morató-Manaro, J., and A. Albrieux: *Endocrinology* 24: 518. 1939.
24. Mühlbock, O.: *Acta brev. Neerland.* 10: 10. 1940.
25. van Heuverswyn, J., S. J. Folley and W. U. Gardner: *Proc. Soc. Exper. Biol. & Med.* 41: 389. 1939.
26. Zondek, B.: *Lancet* 1: 842. 1936.
27. Deanesly, R., and A. S. Parks: *J. of Endocrinology* 2: 487. 1941.
28. Roberts, E.: *J. Biol. Chem.* 118: 627. 1937.

CIRCULATORY EFFECTS OF DIETHYLSTILBESTROL IN CATS AND RATS WITH A NOTE ON INTESTINAL ABSORPTION

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THE PRESENT REPORT deals with the changes in blood pressure and heart rate induced by diethylstilbestrol administered intravenously and by intestinal absorption. The data show that the amount of diethylstilbestrol necessary to change the blood pressure is greater than the largest doses used for estrogenic action.

MATERIALS AND METHODS

Twenty-two cats and 20 rats were used. Approximately one-half of the cats were females, whereas all of the rats were castrated females. The experiments were acute and lasted as long as 6 hours for the cats but not more than 30 minutes for the rats. The animals were anesthetized with urethane and nembutal. Four cc. per kg. of a 25 per cent solution of urethane was given intramuscularly, and 0.4 cc. of nembutal solution (1 grain per cc.) given intraperitoneally. The blood pressure was determined by a mercury manometer. The manometer used for rats was smaller in diameter than the usual type. The blood pressure was always taken from the carotid artery in the cat experiments and from either the carotid or the abdominal aorta in rats. In almost all of the cats plethysmograph records of the leg were obtained simultaneously with plethysmographic recordings from either the liver, intestine, or kidney. In some experiments three plethysmographs were used at one time.

The diethylstilbestrol[1] was given either as a suspension in physiological salt solution or in solution in weak alkalie. The latter was prepared by dissolving 20 mg. of diethylstilbestrol in 0.5 cc. of N NaOH. Both solutions were used in concentrations of 1 to 5 mg. per cc. and prepared fresh as needed.

RESULTS

Intravenous injection. Diethylstylbestrol, given to cats intravenously in doses from 0.1 to 2.5 mg. per kg. of body weight brought about a slight fall in blood pressure which was not related to the size of the dose. Table 1 contains the data from 53 experiments in which diethylstilbestrol was given in suspension and 37 in which the sodium salt was used. These data were subdivided and studied according to the dosage level. This analysis revealed no more than that of the combined group, namely that diethylstilbestrol has only a weak depressor action on blood pressure and no effect on heart rate. In 9 control experiments (*group B*) normal salt solution was given intravenously in amounts similar to those used in 53 experiments in which diethylstilbestrol was given in suspension. A rise in blood pressure of two mm. of Hg in these

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[1] The diethylstilbestrol was supplied by The Schering Corp. Inc., Bloomfield, N. J.

controls confirms the validity of the observation of the slight fall in blood pressure (− 13 mm.) induced by diethylstilbestrol (table 1). The significance of the fall in blood pressure was also revealed by statistical methods. The difference of the means divided by the standard error of the difference was obtained; if this figure was 3 or more, the means were considered to be significantly different (table 1). Since these data representing blood pressures before and after administration of diethylstilbestrol are in pairs, we have used the tables of Student (1, 2) to determine the odds shown in table 1. This means that the odds are 644:1 (*group A*) against a difference as great as this occurring due to chance alone.

Similar, but less convincing, are the interpretations of the fall in blood pressure

TABLE 1. RESPONSE OF BLOOD PRESSURE AND HEART RATE OF CATS AND RATS TO DYETHYLSTYLBESTROL

Group	Number Experiments	Dosage, per/kg.	Blood Pressure, mm. Hg					Heart Rate Per Minute		
			Before	Effect	Change	Mean Diff. / S E of Diff.	Odds	Before	Effect	Change
Intravenous injection in cats										
A, Experimental	53	0.6 mg.[1]	129	116	−13	3.0	644:1	186	187	+1
B, Control	9	1.0 cc.[2]	117	119	+2			174	175	+1
C, Experimental	37	0.8 mg.[3]	99	84	−15	3.9	9999>1	210	206	−4
D, Control	18	0.8 cc.[4]	108	97	−11	2.8	1065:1	196	198	+2
Intestinal absorption in cats										
E, Experimental	11	6.6 mg.[5]	112	94	−18	3.6	2499:1	204	7198	−6
F, Control	10	2.0 cc.[4]	103	94	−9	0.9	13:1	21	210	−8
G, Experimental	17	7.0 mg.[1]	115	98	−17	3.8	9999>1	205	200	−5
H, Control	7	4.0 cc.[2]	96	93	−3		76:1	199	196	−3
Rat experiments										
I, Experimental	27	0.5 mg.[5]	107	83	−24	7.1	9999>1	315	330	+15
J, Control	14	0.5 cc.[6]	119	112	−7	2.3	207:1	327	316	−11

[1] Diethylstilbestrol in saline suspension.
[2] Saline.
[3] Diethylstilbestrol in alkaline solution.
[4] Alkaline solution.
[5] Diethylstilbestrol intravenously in alkaline solution.
[6] Alkaline solution intravenously.

Standard error of Difference $= \sqrt{(SE_1)^2 + (SE_2)^2}$

$SE =$ *Standard error of Mean* $= \frac{SD}{\sqrt{N}}$

$SD =$ *Standard Deviation* $= \sqrt{\frac{\Sigma d^2 f}{n}}$

brought about by diethylstilbestrol in alkaline solution. The data from 37 such experiments are summarized in table 1 (*Group C*) as are also those of 18 control experiments (*group D*) in which NaOH was given intravenously in amounts similar to those used in the diethylstilbestrol experiments. A comparison of these results reveals the fact that the blood pressure fell 15 mm. in the animals receiving diethylstilbestrol and 11 mm. in the controls, or a difference of only 4 mm. The heart rates were practically unchanged in each group (*C* and *D*, table 1).

In rats a comparable amount of diethylstilbestrol in alkaline solution brought about a more noticeable fall in blood pressure and the heart rate was increased (*groups I* and *J*, table 1). Thus it is evident that diethylstilbestrol given intravenously has a depressant effect on blood pressure in both cats and rats beyond that expected from alkaline solution. The increased rate of the heart, noted in the rat experiments, may be open to question because of the difficulty of counting the heart rate in rats.

Intestinal absorption. Since the results with diethylstilbestrol given intravenously in aqueous suspension showed only a slight depressant effect on blood pressure, and this reaction was even less clear when the substance was given as the sodium salt, it

seemed evident that further confirmation of the fall in blood pressure was needed. This we have obtained by injecting diethylstilbestrol into the intestinal tract. Immediately following the injection the blood pressure rose, due to the sensory stimulation necessitated by the manipulation of the intestines. In 3 to 5 minutes the blood pressure began to fall and continued downward for about 15 minutes; thereafter it gradually returned to pre-injection level.

Diethylstilbestrol in alkaline solution produced a fall in blood pressure which was twice that obtained with alkaline solution alone, the figures being 18 and 9 mm., respectively (*groups E* and *F*, table 1). This is comparable to a fall of 17 and 3 mm., respectively, when diethylstilbestrol in suspension and saline only were injected into the small intestines (*groups G* and *H*, table 1).

Plethysmographic records. Plethysmographic records were made of *a*) intact leg, *b*) the whole area of the liver, using the method of Griffith (3, 4), *c*) a segment of the intestine, isolated except for the blood vessels and nerves, and *d*) the kidney, using the method recently described (5). These data were obtained following administration of diethylstilbestrol in suspension and also in alkaline solution, usually given intravenously.

TABLE 2. SUMMARY OF PLETHYSMOGRAPHIC RECORDS FROM CATS

Number of Recordings	Structure	Constriction	Dilation	No Change
39	Leg	26	4	9
9	Liver	6	1	2
11	Intestine	1	8	2
32	Kidney	6	19	7

The volume changes in the leg, liver, intestine and kidney are indicated in table 2, as are the number of experiments in each case. From these data it is apparent that the leg and liver usually constricted whereas the intestine and kidney dilated. Although the reduced volume noted in the leg and liver may have been passive, due to the fall in blood pressure, there were some records which showed that the constriction was under way before the blood pressure began to fall.

DISCUSSION

The depressant effects of diethylstilbestrol are not marked, according to Loeser (6). Our data (table 1) show that even in relatively large doses diethylstilbestrol has only a minor effect on the circulatory system. Therefore, the small fall in blood pressure, as well as the stable rate of the heart, are favorable reactions and indicate that diethylstilbestrol can be safely given in smaller doses for its estrogenic action without fear of disturbance to circulation. Also of interest is the fact that the fall of blood pressure following the administration of diethylstilbestrol was of short duration, and even after repeated doses the return to the pre-existing value was satisfactorily established within a few minutes, thus differing from depressor agents such as amyl nitrite and histamine.

It is interesting to see the fall in blood-pressure following an injection of diethylstilbestrol into the lumen of the small intestines. The time elapsing before absorption began, as determined by the fall of blood pressure, was the same for either the aqueous suspension or the sodium salt of diethylstilbestrol. This is interesting in light of the insolubility of diethylstilbestrol in water and leads one to wonder if the alkali of the succus entericus is sufficiently strong to form soluble salts of diethylstilbestrol quickly.

It seems more likely that diethylstilbestrol was absorbed uncombined with alkali. This view is supported by the solubility of diethylstilbestrol in fats and by the fact that we have found that suspensions of diethylstilbestrol in 1/20 N HCl were readily absorbed from the intestinal tract.

SUMMARY

The data from cats and rats are consistent in showing that diethylstilbestrol has a slight depressant effect on blood pressure. This is usually of brief duration and is followed by a return to the previously existing level. The amount of diethylstilbestrol per kg. of body weight necessary to induce a change in blood pressure is far greater than the dosage administered for estrogenic action.

The rate of the cat's heart was not affected by diethylstilbestrol.

Plethysmographic records indicate constriction of the liver and leg and dilatation of the intestine and kidney.

Absorption from the intestinal tract was evident in as short a time as 3 minutes and lasted for about 15 minutes. As far as could be determined these values were the same for diethylstilbestrol in suspension and in solution as for the sodium salt.

REFERENCES

1. STUDENT: *Biometrika* 6: 1. 1908.
2. LOVE, H. H.: *J. Am. Soc. Agronomy* 16: 70. 1924.
3. GRIFFITH, F. R., J. YORK AND A. ZACHMYS: *Proc. Soc. Exper. Biol. & Med.* 25: 399. 1928.
4. GRIFFITH, F. R., AND F. E. EMERY: *Am. J. Physiol.* 95: 20. 1930.
5. EMERY, F. E.: *J. Lab. & Clin. Med.* 28:208. 1942.
6. LOESER, A., AND K. ERBACHER: *Ztschr. f. d. ges. exper. Med.* 105: 430. 1939.

EFFECTS OF TESTOSTERONE PROPIONATE ON THE MAMMARY GLANDS OF FEMALE ALBINO RATS[1]

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In previous communications from this laboratory (1, 2) it was reported that the action of parenterally administered testosterone propionate on the ovaries of female rats varied according to the phase of the estrous cycle at which the first injection was given. When given during late diestrus the ovaries were small and contained many follicles in various stages of development but they lacked recent corpora lutea. The uteri were enlarged and the endometria hyperplastic. The vaginae were mucified, and the mammae presented evidence of duct proliferation with a marked increase of fibrous tissue. There was, however, no evidence of acinar growth. Similar organ changes, with the exception of the ovaries, were found in castrates treated with testosterone (3).

When the initial injection was given during late estrus the ovaries were distinctly enlarged, due to the presence of hypertrophied corpora lutea and follicles in various stages of development. The uteri of these animals showed a weight increase but there was no endometrial proliferation. The vaginae were in an advanced state of mucification and many epithelial cells were vacuolized, and the acini of the mammary glands were in a state of intensive proliferation. It was assumed that the changes in the vaginal and mammary tissues of the rats treated during estrus resulted from an increased corpus luteum function, and it was demonstrated that deciduomata could be induced in traumatized uteri in the presence of hypertrophied corpora lutea (4).

It is well known that in rats about to litter the mammary glands are completely prepared for lactation, as evidenced by acinar proliferation. However, secretion is present in only moderate amounts. Almost the same picture is encountered in rats which have been given testosterone parenterally during estrus for 20 days. It is furthermore accepted that the suckling stimulus sets off the mechanism of lactation. Since mammary tissues can be brought into a pre-lactating state by testosterone, it was considered of interest to see whether this type of mammary gland would respond to the influence of suckling, and the present report deals with two experiments regarding this problem.

METHODS

A total of 73 adult females of the Stanford strain of albino rats was used. Their ages varied between 110 and 155 days at the beginning of the experiments. All rats treated with testosterone were virgin females and were observed daily by vaginal smears for at least 28 days prior to the first injection. Only females with estrous cycles of from 4 to 6 days were included. They were fed Tioga chow and allowed tap water freely. They were housed in groups of 4 to 5 per cage.

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All treated rats were given the initial subcutaneous injection during late estrus, *i.e.*, when the vaginal cornification had been present for the previous 15 hours. The preparation used was testosterone propionate[2] in oil, in concentrations of 10 and 25 mg. per cc. The controls received plain sesame oil in amounts equal to the amounts used in the treated animals. Individual and total dosages, as well as periods of treatment, are reported.

For comparison, pregnant and lactating females of corresponding ages were chosen from our breeding stock and kept under identical housing conditions, except that they were kept in individual cages.

Group I. Nineteen females received a total of 40 mg. of testosterone. Of these, 4 were killed after 16 days; 4 after 18 days; and 11 on the 21st day. The same number of control animals received 0.4 cc. of plain sesame oil and were killed on corresponding days.

Group II. Six females, which had been under observation since weaning, received 2.5 mg. of testosterone every other day for a period of 20 days, a total dosage of 25 mg. After the eighth injection the rats were placed in individual cages. Twenty-four hours following the last injection, 4 to 6 sucklings 6 to 10-days-old were put with each treated rat, and these were replaced by new sucklings every 2 to 3 days in order to maintain a strong suckling stimulus. The behavior of the treated rats toward the young was noted. On the tenth day the young were taken from their 'foster-mothers' and immediately killed and their stomachs inspected for the presence of milk. In addition, a biopsy was taken of the mammary glands of the treated rats and one ovary was removed for examination.

Group III. Fifteen females received 2.5 mg. of testosterone in oil every other day. Of these, 5 were killed after 30 days, the total dosage being 37.5 mg.; from 5 a biopsy of the mammary gland was taken after 48 days, the total dosage amounting to 60 mg.; and the remaining 5 were killed after 60 days, having received a total of 75 mg. of testosterone.

Group IV. Fourteen female rats of our breeding stock were selected and put with males. The day of successful mating was determined by the presence of spermatozoa in the vaginal content; they were then separated and the females placed in individual cages. Biopsies were taken of the mammary glands and one ovary removed, as follows: from 2 rats on the 16th day of pregnancy; from 2 on the 18th day of pregnancy; from 4 on the day of littering within two hours after the whole litter was born; from 3 on the 10th day of lactation; and from 3 on the 10th day after littering, the young having been weaned immediately after birth.

RESULTS

The administration of testosterone to female virgin rats during late estrus resulted in proliferation of the acini in the mammary glands in a manner comparable to that seen during normal gestation, the growth of the breast tissue being due to the secretion of progesterone from the hypertrophied corpora lutea. Examination of the mammary glands on the 16th and 18th days of treatment, or of pregnancy, revealed numerous collapsed acini (fig. 1, 2) in which the epithelium contained vacuoles believed to be precursors of secretion. On the 21st day all acini had lumina containing occasional secretory material (fig. 3, 4).

Of the 6 rats treated with testosterone for 20 days and then exposed to vigorous suckling, 4 successfully nursed on the second and third days. On the fourth and fifth

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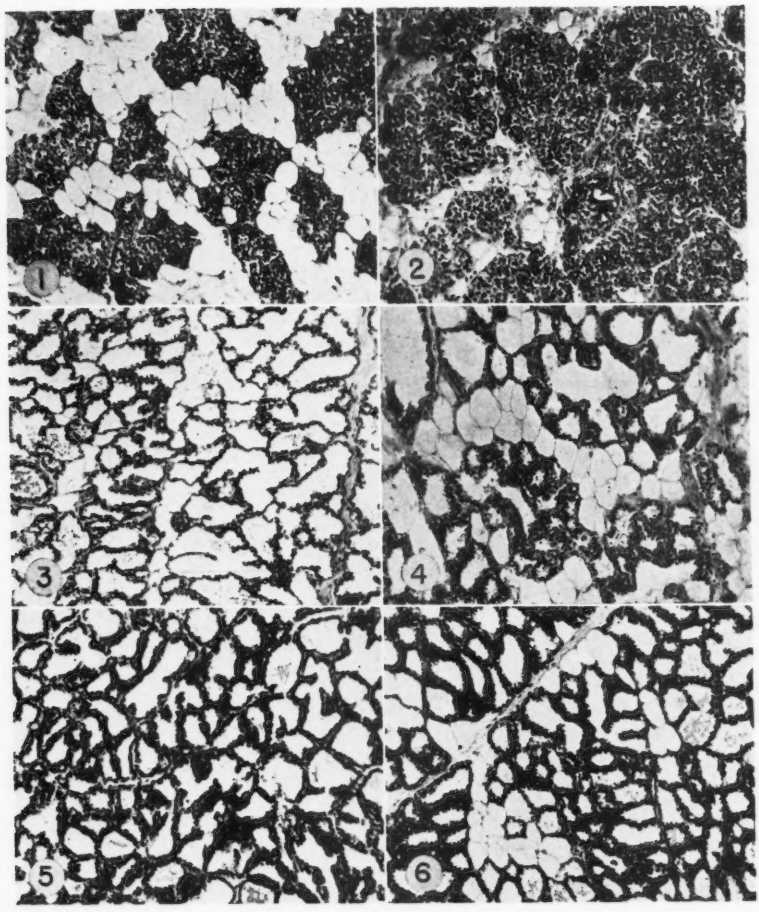
[2] The testosterone propionate was generously supplied by the Ciba Pharmaceutical Products, Inc., Summit, N. J.



MAMMARY GLANDS OF FEMALE RATS. Fig. 1. Sixteenth day of normal pregnancy. Fig. 2. After 40 mg. of testosterone in 16 days.

Fig. 3. On day of littering (21st day of normal pregnancy). Fig. 4. After 40 mg. of testosterone in 21 days.

Fig. 5. On 10th day of normal lactation.

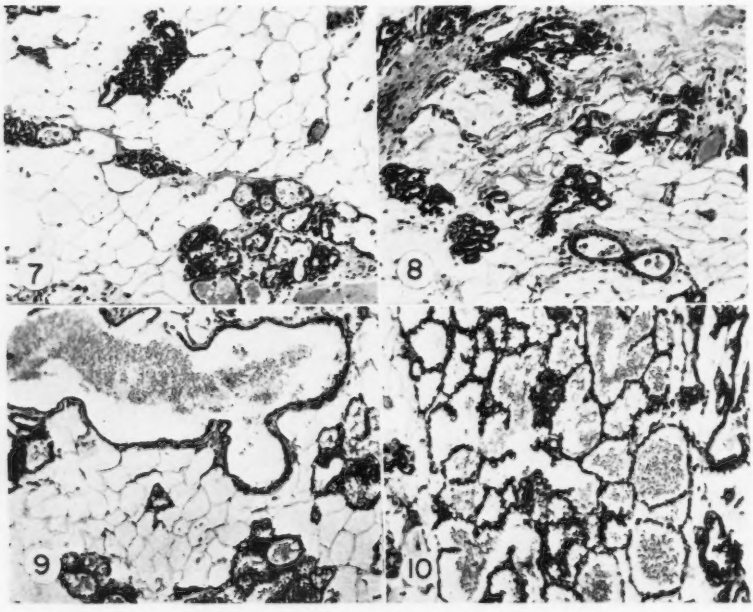
Fig. 6. After 25 mg. of testosterone in 20 days, followed by nursing for 10 days.

days the nipples and surrounding areas assumed the appearance of those of normal nursing rats. Two animals began building nests a few hours after the young were placed with them, while the other 2 nested on the fourth day. From the fourth day on, they exhibited all the characteristics of the mother rats of our colony. They tried to prevent the removal of the young, and when a fresh litter was placed in the front part of the cages they hurriedly carried them to the nests and promptly began nursing them. Inspection of the stomachs of the sucklings on the 10th day revealed the presence of milk in 8 of the 20 which had been nursed during the previous 48 hours. It should be noted, however, that the distention of the stomachs was less than that seen

in those nursed by normal mothers. To make certain that the milk found was not from previous nursing of normal mothers, 9 young rats of the same age were separated from their mothers and their stomachs inspected the next day. No milk was found in any of them 17 hours after they had been weaned.

Microscopic examination of the lactating glands of these 4 foster mothers revealed no difference from those seen in untreated lactating rats (fig. 5, 6).

The two rats which failed to foster had mammary glands closely resembling those found in rats not allowed to nurse after littering. They showed nests of acini undergoing involution, separated by masses of fatty tissue (fig. 7, 8).



MAMMARY GLANDS OF FEMALE RATS.. Fig. 7. On 10th day after littering; nursing not allowed. Fig. 8 After 25 mg. of testosterone in 20 days followed by unsuccessful nursing for 10 days.
Fig. 9. After 37.5 mg. of testosterone in 30 days. Fig. 10. Mammary gland after 75 mg. of testosterone in 60 days.

When the injection of testosterone was continued beyond the 20-day period an increase in secretory changes within the acini became evident. Comparative histologic studies of the ovaries of rats treated at various dosage levels indicated that the previously hypertrophied corpora lutea regressed in spite of continued hormone administration, leading ultimately to their disappearance. While regressive changes took place in the corpora, there was a progressive increase in acinar secretion in the mammary glands, so that the largest amount of secretion was found when the corpora lutea were absent (fig. 9, 10). The initiation of secretion, therefore, was due mainly to the regression of the corpora and not to the increase in length and total dosage of testosterone.

DISCUSSION

The effects of testosterone on the mammary glands of virgin adult rats can be better understood when the ovarian changes are taken into consideration. Reece and

Mixner (5) and Meites and Turner (6) report that testosterone increases the production of lactogenic hormone in the anterior hypophysis. Evans and his associates (7, 8) also found that the lactogenic hormone of the anterior hypophysis was the only pituitary hormone capable of maintaining the function of corpora lutea in the hypophysectomized rat.

On the basis of our findings and the above reports, the following theory can be postulated. When the initial administration of testosterone propionate occurs during late estrus the hormone stimulates the production of hypophysial lactogenic hormone, which in turn maintains the function of the newly formed corpora lutea (1, 2). As one of the various effects of continued secretion of progesterone, the acini of the mammary glands develop, but there is no evidence of lactation. However, the addition of a suckling stimulus acting on the morphologically prepared breast tissue produces copious milk secretion. This conforms with the general experience that continuation of adequate lactation can be maintained only by a persistent mechanical stimulus on the nipples. Experimentally, this was confirmed by Selye (9, 10) in rats. It also has been shown recently that the suckling stimulus *per se* is necessary for maintaining lactogenic hormone production in the anterior hypophysis (6, 11, 12, 13). From the above, it may be deduced that the suckling stimulus maintained lactogenic hormone production in our rats after the administration of testosterone was discontinued.

The fact that the male hormone did not abolish the maternal instinct was surprising. However, it is possible that the secretion of lactogenic hormone, stimulated by testosterone propionate and consecutively maintained by the suckling stimulus, preserved the maternal instinct. Support for this hypothesis is seen in the findings of Riddle, Lahr and Bates (15) who report that prolactin was the only pituitary hormone which induced maternal behavior in 60 to 80-day-old virgin rats.

The initiation of lactation which followed continuation of treatment appears to be closely connected with regressive changes in corpora lutea. This interrelationship between ovaries and mammary glands is particularly impressive after the longer periods of hormone administration when corpora have completely disappeared and secretory changes in the breast tissue are most pronounced. In early phases of treatment the reverse is found, *i.e.*, the corpora lutea are functioning and there is no evidence of lactation. In this connection, the recent report of Meites and Turner (14) is of importance. These authors demonstrated that lactation was suppressed during normal gestation because large amounts of progesterone inhibited the action of estrone on the anterior hypophysis and thus prevented stimulation of lactogenic hormone in sufficient quantities.

It is therefore of interest that a pre-lactating phase can be induced by testosterone when given during estrus, and that this phase can be transformed into true lactation in an animal not previously pregnant by the mechanical stimulus of suckling.

SUMMARY

Testosterone propionate injected into virgin female rats during late estrus produces development of the acini in the mammary glands within 20 days. It is believed that this growth is caused by the secretion of progesterone from the hypertrophied corpora lutea. Such mammary glands are capable of lactating when exposed to a suckling stimulus. Copious milk production and successful fostering of 6 to 10-day-old rats has been demonstrated.

Continuation of treatment beyond 20 days results in a regression of hypertrophied corpora lutea, which is accompanied in the mammary glands by a marked increase in stored secretion resembling milk. The secretion increases with the regression of the corpora lutea.

REFERENCES

1. Laqueur, G. L., and C. F. Fluhmann: *Endocrinology* 30: 93. 1942.
2. Schilling, W., and G. L. Laqueur: *Endocrinology* 30: 752. 1942.
3. Laqueur, G. L.: *Proc. Soc. Exper. Biol. & Med.* 49: 425. 1942.
4. Fluhmann, C. F., and G. L. Laqueur: *Endocrinology* 31: 375. 1942.
5. Reece, R. P., and J. P. Mixner: *Proc. Soc. Exper. Biol. & Med.* 40: 66. 1939.
6. Meites, J., and C. W. Turner: *Endocrinology* 30: 711. 1942.
7. Evans, H. M., M. E. Simpson, and W. R. Lyons: *Proc. Soc. Exper. Biol. & Med.* 46: 586. 1941.
8. Evans, H. M., M. E. Simpson, W. R. Lyons, and K. Turpeinen: *Endocrinology* 28: 933. 1941.
9. Selye, H.: *Am. J. Physiol.* 107: 535. 1934.
10. Selye, H.: *Anat. Rec.* 60: 323. 1934.
11. Reece, R. P., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 35: 367. 1936.
12. Reece, R. P. and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 35: 621. 1936.
13. Meites, J., A. J. Bergman, and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 46. 670. 1941.
14. Meites, J., and C. W. Turner: *Endocrinology* 30: 719. 1942.
15. Riddle, O., E. L. Lahr, and R. W. Bates: *Proc. Soc. Exper. Biol. & Med.*, 32: 730. 1935.

TECHNIQUE FOR THYROIDECTOMY IN THE PIGEON AND THE EARLY EFFECT OF THYROID REMOVAL ON HEAT PRODUCTION

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ALTHOUGH thyroidectomy in fowl (1–7), geese (8), ducks (1) and pigeons (9, 10) has been reported, the descriptions of the technique for these operations have been found by us to be quite inadequate. The pigeon has many advantages for the study of calcemia, lipemia, and certain aspects of carbohydrate and fat metabolism, but recognition of the rôle of the thyroid in these processes has been retarded by the difficulty of obtaining large numbers of thyroidectomized birds with unimpaired digestive apparatus. The technique here described has been found to be satisfactory in this respect.

MATERIALS

White Carneau pigeons about 2 months old have been used in this study. The bird is fastened on its back to the operating board by rubber strips cut from automobile inner tubes. One strip on each side holds each wing and a strip across the end holds the feet in place. The board is placed on the operating table with the bird's head toward the operator and the proximal end is supported about two inches higher than the opposite end. Using such a small board (30×36 cm.) the bird's position may be readily adjusted.

Retractors are made of wire bent to form a hook on one end and an eyelet on the other. Rubber bands are attached to the eyelets and to movable glass-headed pins on the board to furnish the desired tension. The instruments required consist of the following: 2 fine forceps with curved points and long handles, 2 fine (iridectomy) forceps with straight points and short handles, one fine-pointed curved scissors, 2 or 3 hemostats with straight slender points, a small (no. 7) half-circle suture needle, and fine thread or gut. Cotton pellets[2] of various sizes complete the necessary materials.

Nembutal gives highly satisfactory and durable anesthesia. The bird, previously fasted about 10 hr., is given the anesthetic intravenously on a standard dose of 25 mg. per kg. of body weight. The injection is made *slowly* into the radial vein which is exposed by the plucking of a few feathers.

OPERATIVE PROCEDURE

Exposure of the two glands. The left thyroid is removed first and the right is removed not earlier than 4 days after the first. The feathers are plucked from the throat in the region to be incised. The incision is approximately 5 cm. long, paralleling the edge of the furcula but lateral to the crop (ingluvies). In figure 1 the positions of the

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[2] Richmond Dental Mfg. Co., Niagara Falls, N. Y.

incisions and the extent of the plucked area are shown, for convenience, as if both glands were to be removed at the same time.

The lateral edges of the crop are freed from the connective tissue and the free lobe of the crop is then pulled to the midline. Using blunt dissection the connective tissue is torn between the crop and the furcula and the crop then retracted with the wire hook. A small band of muscle tissue, the position of which varies somewhat, connects the medial edge of the furcula and the crop. This band must not be cut. At this stage the rose-red gland can be seen through the transparent wall of the air sac. Although it was thought inadvisable by Vermeulen (11), the air sacs can be torn away and free access to the gland obtained. The relationships of the anatomical structures at this stage are shown in figure 2.

It can be seen that anterior to the thyroid the jugular vein is held tightly to the trachea by connective tissue. Tearing this connective tissue with forceps will allow necessary mobility such that the trachea can now be included under the hook of the right retractor.

A small vein is found connecting the thyroid and the trachea (see fig. 2). It is doubly ligated and cut between the ligatures. This allows the thyroid to be rotated to the left to uncover the arterial supply at the medio-caudal region (see fig. 3). The thyroid artery arises as a branch of the cervical artery and is embedded in connective and fatty tissue (see fig. 3 and 4). The connective tissue is torn away and the cleaned artery is doubly ligated and cut between the ligatures. Some difficulty may be encountered in placing the suture around the artery. This can usually be done most easily by using the long curved forceps, held as a pencil, with the points curving upward. The points are hooked under the artery so that the tips project behind it. By working the tips against those of another forceps, the tissue can be pierced; the hole thus made is enlarged by allowing the forceps to spread somewhat.

The gland at this stage is broadly attached only to the jugular vein. At each end the gland is separated slightly from the vein to form notches which will help to hold the ligature in place (see fig. 5). While the ligature is gradually tightened the gland is pulled farther through the loop so that none is retained in the knot. The knot is tied very tightly, and for this purpose hemostats can be used more effectively than forceps. The gland is then held with the forceps and cut away, care being taken to cut as close to the knot as possible. By this method the left jugular vein remains intact and unobstructed.

The exposed surfaces are moistened occasionally with physiological saline during the operation and while the incision is being closed. The skin over the crop is now pulled back over the furcula and the incision closed with a continuous suture. Although the birds recover from the anesthesia in a few hours, they should be kept for 12 to 24 hours without food but with access to water.

The exposure of the right thyroid is accomplished in the same way as in the case of the left. The jugular vein, anterior to the thyroid, is separated from the vagus nerve and the cervical artery by blunt dissection. The jugular is then doubly ligated and cut between the ligatures. The thyroid is grasped at its anterior end, lifted upward (ventrally) and dissected (caudally) free of the nerve and artery (see fig. 6). Occasionally a small branch from the cervical artery feeds the anterior end of the thyroid but this branch can be safely torn with no more than temporary bleeding.

At this point a vein, varying somewhat in position, is seen connecting the medial edge of the thyroid with the crop. This is doubly ligated and cut between the ligatures. This allows the blunt dissection to proceed until the gland is attached to the bird by only the jugular vein and the thyroid artery. Care should be taken to avoid

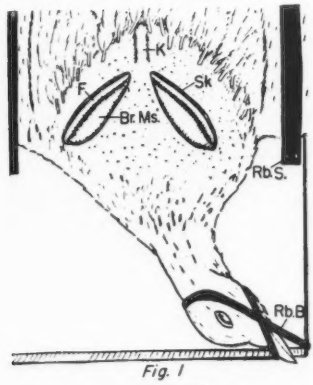

Fig. 1

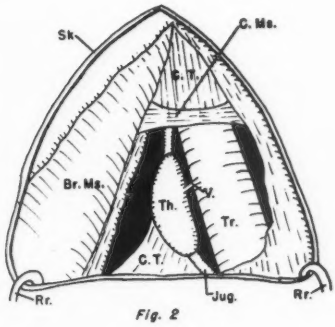

Fig. 2

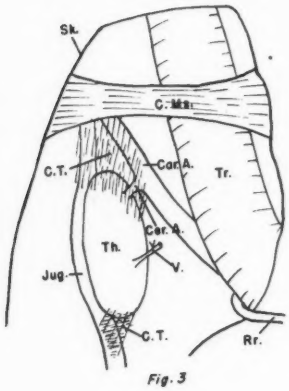

Fig. 3

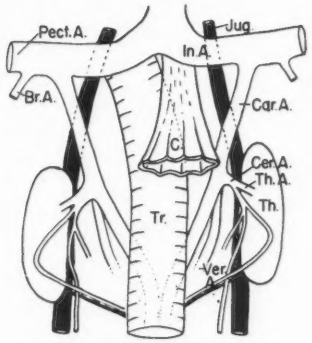

Fig. 4

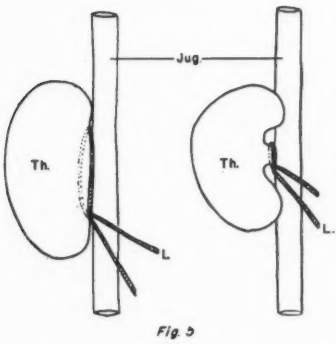

Fig. 5

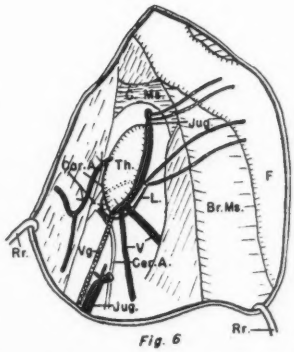

Fig. 6

Fig. 1–6. STAGES IN THE REMOVAL OF THE THYROIDS OF PIGEONS. Key: *Br. A.*, brachial artery; *Br. Ms.*, breast muscle; *C.*, crop; *C. Ms.*, crop muscle; *C. T.*, connective tissue; *Car. A.* carotid artery; *Cer. A.*, cervical artery; *F.*, fat; *In. A.*, innominate artery; *Jug.*, jugular vein; *K.*, keel; *L.*, ligature; *Pect. A.*, pectoral artery; *Rb. B.*, rubber band; *Rb. S.*, rubber strap; *Rr.*, retractor; *Sk.*, skin; *Th.*, thyroid; *Th. A.*, thyroid artery; *Tr.*, trachea; *V.*, vein; *Ver. A.*, vertebral artery; *Vg.*, vagus. In figure 4 the thyroids have been rotated laterally.

tearing the veins entering the jugular at the caudal end of the thyroid (see fig. 6). A ligature is placed over the anterior end of the gland, slipped caudally until it encircles only the two blood vessels, and then tied. The gland is cut free immediately anterior to the ligature. The incision is then closed and the bird allowed to recover from the anesthetic as described above.

Post-operative care. The birds usually recover uneventfully, although an occasional one becomes crop-bound. The incidence of crop-binding is reduced by removing the gland in two stages and by avoiding undue exposure and drying of the tissues. It is thought advisable to restrict the diet to small grains, avoiding uncracked corn, peas and beans. The animals should be kept at a reasonably uniform temperature.

Discussion of technique. Postmortem examinations show that the left jugular is relatively unaffected by the removal of the left thyroid. The right jugular below (cardiad to) the final ligature is usually distended but there is evidence that some collateral vessels develop. Both glands have been removed from a few birds by tying off the jugular veins above and below the glands and removing a section of the jugular with the gland attached. The birds survive the ligation of both jugulars, apparently because of their marked ability to develop collateral pathways rapidly, but the impeded venous return from the head is probably not without effect.

Early in the development of the technique, it was found that cautery alone was not practical because of resulting hemorrhages and proximity of the glands to the vagi. It is thought advisable, however, to cauterize[3] the ligated stump after removal as a routine precaution against regeneration of thyroid tissue.

Results obtained earlier in this laboratory from standardized feeding in conjunction with other experiments on thyroidectomized pigeons, indicate that the elimination of food from the crop is slower than in normal birds. In view of the fact that Lieberfarb (12) found an increased crop motility in fowl fed thyroid substance, one might expect the converse to be true in thyroidectomized pigeons. Observations on the body weights of 15 thyroidectomized birds for 8 to 25 days indicate an average loss of 2.8 gm. per day for the first 10 days after the operation. At 15 days after thyroidectomy, the body weights were essentially stationary and remained so for the rest of the observation period.

Macroscopic examination of the birds at autopsy usually failed to reveal any visible remnants of thyroid tissue. In a few cases suspected of having residual thyroid tissue, the tissue immediately enclosing the final ligature on each side was removed and prepared for histologic study. Microscopically the material showed the expected connective tissue infiltration and encapsulation. In only 3 cases were remnants of thyroid tissue found. Preliminary information on the completeness of removal can be obtained at the time of the operation by examining the extirpated gland; the smooth surface and spheroidal shape of the completely removed gland is quite apparent.

EARLY EFFECT ON HEAT PRODUCTION

Some determinations of basal heat production have been made on birds soon after their operation with the above-described technique. The method of Benedict and Riddle (13) was used on the unoperated, half-thyroidectomized and completely thyroidectomized pigeons. In accord with this method the birds were uniformly fed 15 gm. of food at the beginning of a 24-hr. fast. While fasting the birds were maintained at a temperature of 30° C. and the determination itself carried out at this same temperature.

One group of 6 birds had a basal heat production of −10 at 4 to 5 days after the

[3] Post Illuminated Cautery set, Post Electric Co., Andover, N. J.

removal of one thyroid, but probably part of this reduction was a result of proximity of the determination to the previous (operative) fast. At 7 to 8 days after the removal of the second thyroid the heat production had been further depressed to −19. In a second group of 8 birds completely thyroidectomized for 6 to 7 days, the heat production was −21 when compared to the value at 4 to 5 days after half-thyroidectomy. Unoperated control values were not obtained for this group. In a third group the postoperative periods varied to a much greater extent. Three birds when half-thyroidectomized for 5 to 19 days had heat production values of −4 as compared to controls. When completely thyroidectomized for 6 to 46 days the heat production of 7 birds was −27. This value is in satisfactory agreement with additional data (by Riddle and Smith, unpublished) obtained in this laboratory. It may be noted that an average respiratory quotient of 0.71 was obtained in 14 operated birds.

SUMMARY

The thyroids were removed from pigeons by a two-stage operation. The technique described avoids the immobilization of the crop ('crop-binding') which is frequent when other methods are used, and thus increases the usefulness of the birds for certain metabolic studies. The basal heat production of 8 birds was apparently depressed within 4 to 5 days after the removal of one thyroid, and in 13 birds it was −20 at 7 to 8 days after complete thyroidectomy; in another group of 7 pigeons a value of −27 was obtained at 6 to 46 days after complete thyroidectomy. Nearly all birds survive this operation and the technique permits a rapid and almost uniformly complete removal of the thyroids of pigeons.

The authors wish to express their appreciation to Dr. Oscar Riddle for materials required for this study and for helpful advice in the preparation of the manuscript.

REFERENCES

1. Benoit, J., and M. Aron: *Compt. rend. Soc. de biol.* 116: 221. 1934.
2. Bomskov, C.: *Methodik der Hormonforschung* 1: 148. 1937.
3. Greenwood, A. W., and J. S. S. Blyth: *Proc. Roy. Soc. Edinburgh* 49: 313. 1929.
4. Parkes, A. S., and H. Selye: *J. Genetics* 34: 297. 1937.
5. Schwarz, E.: *Arch. f. Entwcklngsmechn. d. Organ.* 123: 1. 1930.
6. Taylor, L. W., and B. R. Burmester: *Poultry Sci.* 19: 326. 1940.
7. Winchester, C. F.: *Endocrinology* 24: 697. 1939.
8. Parhon, C. J., and C. Parhon: *Compt. rend. Soc. de biol.* 91: 765. 1924.
9. Ewald, J. R., and J. Rockwell: *Pflüger's Arch. f. d. ges. Physiol.* 47: 160. 1890.
10. Riddle, O., and G. C. Smith: *J. Pharmacol. & Exper. Therap.* 55: 173. 1935.
11. Vermeulen, H. A.: *Arch. Neerland. Sci. Exactes et Nat., Series IIIC,* 13: 603. 1928.
12. Lieberfarb, A. S.: *Ztschr. Vergleich. Physiol.* 10: 751. 1929.
13. Benedict, F. G., and O. Riddle: *J. Nutrition* 1: 497. 1929.

EVIDENCE AGAINST A PROGESTERONE-LIKE ACTION OF ASCORBIC ACID

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Ascorbic acid is known to be present in high concentration in the corpus luteum, and to be increased in concentration in the uterus during progestational changes, due to the effect of progesterone (1). It is also stated that lowered concentration of ascorbic acid in the guinea pig can prevent proper progestational proliferation or, after it has taken place and pregnancy has supervened, removal of this substance from the diet can cause a termination of the pregnancy (2). This evidence led Israel and Meranze to suspect that perhaps vitamin C is associated with progesterone in bringing about progestational proliferation and that alone it may be able to cause similar changes (3). They have published an article (1941) in which they report the results of experiments indicating that vitamin C has this effect. The present work was undertaken as a repetition of their investigation.

The experiments of Israel and Meranze were first repeated exactly as reported in their article. Infantile rabbits, weighing from 600 to 800 gm. at the start of the experiment, were used, since they had been found to give the best results. The vitamin, in the form of monoethanolamine salt of ascorbic acid[1] was used, and the exact method of priming with estrogen and injection of ascorbic acid as employed by Israel and Meranze was repeated. Five thousandths of a mg. of estradiol dipropionate was given daily for 7 days for priming; 500 mg. of the vitamin preparation was injected subcutaneously daily for the succeeding 5 days; the animals were killed on the sixth day.

As the results appeared to be negative, other tests were made with varying dosages, and with a different preparation of the vitamin. Pure crystalline ascorbic acid was used in the following series of experiments: 2 rabbits were given 200 mg. per day for 5 days; 2, 300 mg. per day for 5 days; 2, 500 mg. per day for 5 days; and 2, 500 mg. per day for 10 days.

Control experiments were performed using the same preparation of estrogen for priming and the same strains of animals. With the standard dosage of progesterone,[2] the usual 'three plus' reaction was obtained (fig. 5). An equal number of animals was primed with estrogen, left alone for 5 days and then autopsied with the others. For the 10-day experiments there were two controls receiving no injections for 10 days before autopsy.

At autopsy the uteri varied somewhat in size, those of the progesterone controls being consistently larger than those of the estrogen controls and those of the animals

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[1] The monoethanolamine salt of ascorbic acid (Cenolate) was, in part, obtained locally, the remainder being furnished by the Abbott Laboratories, North Chicago, Ill., which likewise supplied the pure crystalline ascorbic acid.

[2] The progesterone used was supplied by Dr. Erwin Schwenk of the Schering Corp., Bloomfield, N. J.

given ascorbic acid. There was, however, no consistent difference between the two latter groups.

Microscopically, no significant differences were found between estrogen controls and the rabbits given ascorbic acid. This is illustrated by the comparison (see legends) of figures 1 and 3 with figures 2 and 3, and figure 6 with figure 7. The effect of a small

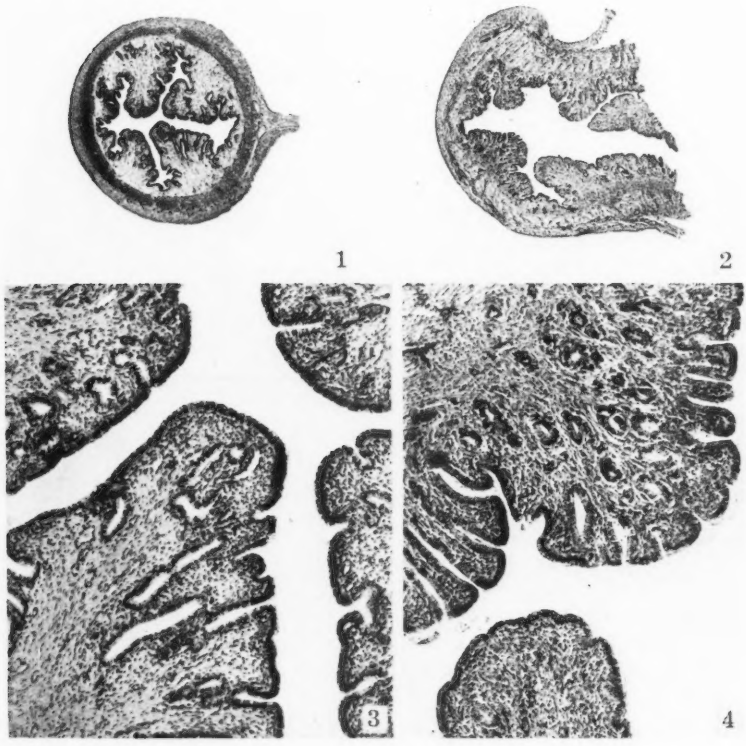

Fig. 1. Uterus of rabbit given 500 mg. of ascorbic acid per day for 10 days after preparation with estrogen. ×10. Fig. 2. Control uterus on experiment illustrated in figure 1. Prepared with estrogen and autopsied 10 days later. ×10.

Fig. 3. Same uterus as in figure 1 under higher magnification. ×60. Fig. 4. Same uterus as in figure 2, under higher magnification. ×60.

dose of progesterone (.1 mg. daily for 5 days) is shown in figure 5. In one of the animals given 500 mg. per day for 10 days, (fig. 1) there did appear to be definite branching of the glands, but it was hardly more pronounced than in an ordinary estrogen control (fig. 2) and was in no way like even a 1 plus progesterone reaction.

In one of the animals given 300 mg. of ascorbic caid daily for 5 days, the endometrium showed a greater number of glands, with more dilatation, than was seen in any of the other experiments. The reaction, however, did not resemble that caused by progesterone, as will be seen in figure 8. This was not a uniform effect of the par-

ticular dosage, as is indicated by figure 9 from the other animal given this same dosage. Probably it was an unusually marked reaction to the estrogen preparation. In any experiment involving the use in sequence of two drugs, as is necessary in the case of

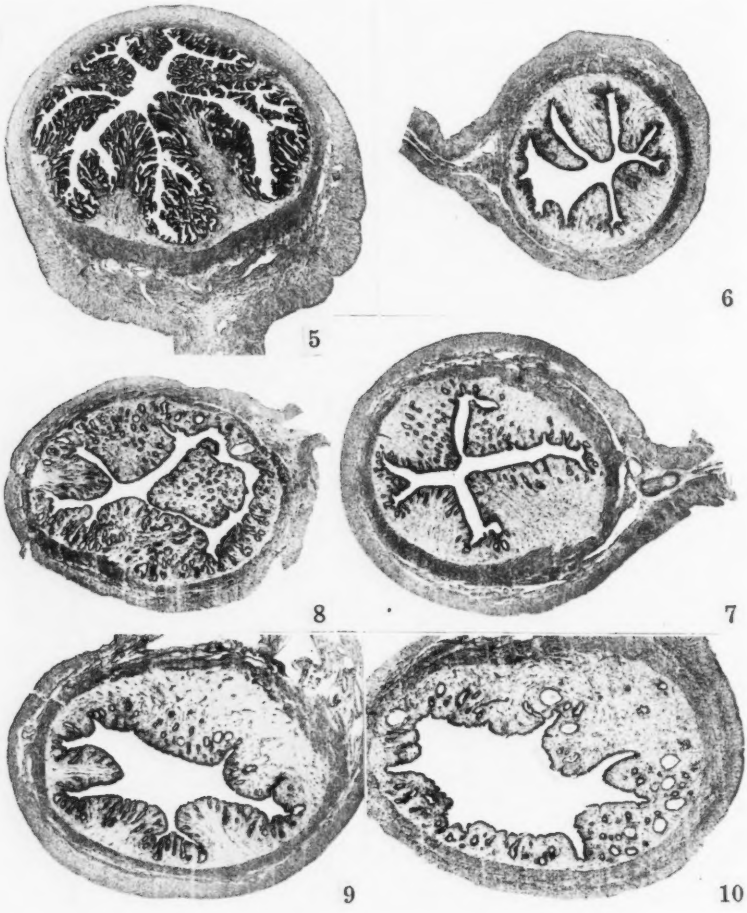

Fig. 5. Uterus of progesterone control rabbit. Prepared with estrogen and given .1 mg. of progesterone per day for 5 days. ×10. Fig. 6. Uterus of rabbit given 500 mg. of ascorbic acid per for 5 days after estrogen preparation. ×10. Fig. 7. Uterus control of rabbit for experiments shown in figures 5, 6, 8, 9, and 10. Prepared with estrogen and autopsied after 5 days. ×10.

Fig. 8, 9. Uteri from two animals given 300 mg. of ascorbic acid per day for 5 days. Note that while figure 8 shows considerable dilatation of the glands, the process is quite unlike that shown in fig. 5. Note also the resemblance of figure 9 to figure 7. ×10. Fig. 10. Uterus of rabbit given local application of 210 mg. of ascorbic acid per day for 5 days, through uterine fistula. Note dilated vessels, and other signs of inflammation, but essential resemblance of endometrium to that of figure 7. ×10.

infantile rabbits, one must remember that an atypical reaction to the first drug will change the appearance of the result at the end of the experiment. No such picture as this occurred when using either larger or smaller doses.

Another series of experiments was performed, employing the direct application of ascorbic acid to the endometrium. This was done in order to give the vitamin as much opportunity as possible to elicit its supposed effects upon the endometrium.

It has been shown that characteristic effects of many hormones can be obtained by local application to the responsive tissues (4, 5, 6) and that usually the maximal effect can be obtained with a much smaller dose than is necessary when the hormone is given by subcutaneous injection. Thus, in the case of progesterone, .02 mg. applied directly can cause a reaction which requires 1 mg. given in .2-mg. doses over a period of 5 days when injected subcutaneously (7).

In the experiment just mentioned, the progesterone, dissolved in .1 cc. of sesame oil, was placed in an isolated segment of the uterus at the time of the operation, using infantile rabbits suitably prepared by previous treatment with estrogen. In our experiments with ascorbic acid this method was necessarily modified. Ascorbic acid is not soluble in oil, and if applied locally in aqueous solution it would be too rapidly absorbed. The substance, moreover, is a sufficiently strong acid to be harmful when applied directly to the tissues.

The method used was to make a uterine fistula according to the method of Reynolds (8), by which the two uterine cervices are made accessible by attachment to the abdominal wall.

Ascorbic acid in aqueous solution was injected into the left horn of the uterus through the cervix 3 times daily for 5 days, leaving the right horn as a control. Immediately before each injection the solution of crystalline ascorbic acid was neutralized by adding the necessary amount of sodium bicarbonate, *i.e.*, 0.5 gm. to 1 gm. of ascorbic acid (9). The animals used for this part of the experiment were somewhat larger than the others. They weighed about 1000 gm. at the start of the experiment.

Using this technique several experiments were performed: 1 rabbit was given the monoethanolamine salt of ascorbic acid, 100 mg. 3 times daily for 5 days; 1 rabbit was given the same dose for 3 days; 2 rabbits were given 70 mg. of the pure ascorbic acid 3 times daily for 5 days. This is 210 mg. per day, or a total of 1050 mg. per animal. By analogy with the experiments of McGinty, Anderson and McCullough (7), which indicated that a local application is many times as effective as subcutaneous injection, this amount applied locally is no doubt equivalent to many grams of ascorbic acid injected subcutaneously.

At autopsy the uteri of these animals were somewhat larger than in previous experiments, and the lumina were somewhat dilated, but there is no reason to believe that this enlargement had any underlying cause other than the pressure of the injected fluid. The epithelium was low. There was no evidence of increased branching of the uterine glands as is seen in the response to progesterone (fig. 10).

To summarize, in none of these experiments, nor in those described above in which the vitamin was administered by subcutaneous injection, was there any effect upon the uterus equal to the 'one plus' reaction elicited by a dose of progesterone barely above the threshold for minimal effect in the standard test in suitably primed immature rabbits (Clauburg test).

SUMMARY

In a series of rabbits ascorbic acid (vitamin C) was administered subcutaneously, and also locally into the uterus, in varying dosage during periods of 3 to 10 days. Contrary to a previous report no effects similar to those of progesterone were obtained.

This problem was suggested by Dr. George W. Corner, whose advice and assistance have been invaluable in all aspects of the work.

REFERENCES

1. PINCUS, G., AND J. BERMAN: *Am. J. Physiol.* 119: 455. 1937.
2. KRAMER, M., M. HARMON AND A. BRILL: *Am. J. Physiol.* 106: 611. 1933.
3. ISRAEL, S. L., AND D. R. MERANZE: *Endocrinology* 29: 210. 1941.
4. BERGER, M.: *Klin. Wchnschr.* 14: 1601. 1935.
5. LYONS, W., AND H. TEMPLETON: *Proc. Soc. Exper. Biol. & Med.* 33: 587. 1936.
6. JADASSOHN, W., E. UEHLINGER AND W. ZURCHER: *Klin. Wchnschr.* 16: 313. 1937.
7. MCGINTY, D., L. ANDERSON AND N. MCCULLOUGH; *Endocrinology* 24: 829. 1939.
8. REYNOLDS, S. R. M.: *Am. J. Physiol.* 92: 420. 1930.
9. KARRER, P., AND G. SCHWARZENBACH: *Helvet. chim. acta.* 17: 58. 1934.

INACTIVATION OF TESTOSTERONE PROPIONATE IN THE LIVER DURING VITAMIN B COMPLEX DEFICIENCY. ALTERATION OF THE ESTROGEN-ANDROGEN EQUILIBRIUM[1]

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IN PREVIOUS publications (1–3) we have reported that deficiency of the vitamin B complex in female rats seriously impairs the estrogen-inactivating function of the liver, and that this function can be restored by the addition of brewers' yeast to the diet. The investigation presented here is concerned with the effect of vitamin B deficiency on hepatic inactivation of testosterone propionate. Unlike the estrogens, inactivation of this androgen in the liver is not significantly diminished in vitamin B complex deficiency.

It has been shown (4–6) that testosterone propionate and other androgens are normally inactivated in the liver. When pellets of androgen were implanted in the spleens of previously castrated rats so that the absorbed steroid must pass through the liver before reaching the systemic circulation, no androgenic effect could be detected. If the spleen containing the pellet was subsequently transplanted subcutaneously, and after establishment of a collateral circulation, its pedicle ligated, the specific effect became evident. Burrill and Greene (7) and Selye (8) have provided confirmatory evidence that androgens are inactivated in the liver.

EXPERIMENTAL PROCEDURE

Two parallel and independent series of experiments were performed by each of us in different laboratories, one on rats of the Sherman strain, the other on animals of the Long-Evans strain. Virtually identical results were obtained in both series.

In the first series (table 1) adult male rats of the Sherman strain, weighing from 170 to 244 gm., were castrated, and after intervals of 28 or 42 days, to permit atrophy of the seminal vesicles, a pellet of testosterone propionate (weighing from 7.19 to 10.79 mg.) was implanted in the spleen of each. After a brief interval to permit recovery from the operation, some of the animals were placed on a vitamin B-complex-free diet,[2] while others remained on a complete diet. For control purposes a group of untreated castrated animals (without pellets in their spleens) was also placed on a vitamin B-free diet and a similar group was maintained on a normal diet. Finally, a group

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[1] Aided by a grant to one of us (M. S. B.) from the Winthrop Chemical Company, Inc., New York.
[2] Vitamin B-complex-free diet (Smaco): sucrose, 68 per cent; vitamin-free casein, 18 per cent; vegetable oil, 8 per cent; USP salt mixture No. 2, 4 per cent; USP cod liver oil, 2 per cent. We are indebted to Messrs. W. O. Frohring and H. F. Seibert of the Research Laboratories of the S. M. A. Corp., Chagrin Falls, Ohio, for part of the B-free diet used in these experiments.

TABLE 1. EFFECT OF SPLENIC IMPLANTS OF TESTOSTERONE PROPIONATE ON THE WEIGHTS OF SEMINAL VESICLES IN CASTRATE RATS ON NORMAL AND VITAMIN B-COMPLEX-FREE DIETS. FIRST SERIES, SHERMAN STRAIN.

Rat No.	Body Wt. at Castration, gm.	Interval between Castration and Implantation days	Body Wt. at Implantation of pellet gm.	Days on Diet	Body Wt. End of Experiment gm.	Average Amt. Testosterone Propionate Absorbed per Day mg.	Seminal Vesicles Weight mg.
			Group 1, castrated, pellet in spleen, normal diet[1]				
1	206	28	243	56	285	0.105	90
2	170	28	246	56	285	0.125	65
3	195	42	260	42	262	0.105	50
4	210	42	282	42	299	0.112	53
							mean 65
			Group 2, castrated, pellet in spleen, B-free diet				
5	230	28	278	40	189	0.110	110
6	244	42	320	35	178	0.111	95
7	185	28	252	39	134	0.141	90
8	206	42	275	35	176	0.115	87
9	206	28	250	40	152	0.124	87
							mean 94
			Group 3, castrated, no pellet, normal diet				
10	175			84	257		66
11	205			84	289		60
12	230			85	331		52
13	222			85	278		50
14	208			85	286		40
							mean 54
			Group 4, castrated, no pellet, B-free diet				
15	346			43	174		95
16	319			43	170		80
17	190			48	148		41
							mean 72
			Group 5, normal animals, normal diet				
18					316		1430
19					319		1390
20					307		1360
21					318		1110
							mean 1323
			Group 6, normal animals, B-free diet				
22	290[2]			59	124		250
23	280			40	126		180
24	270			59	125		170
25	255			40	130		135
26	244			59	131		120
							mean 171

[1] The control diet in this series consisted of a complete pellet diet with supplements of lettuce and meat several times a week.

[2] Body weight when B-free diet was begun. The animals in groups 4, 5 and 6 were not castrated.

TABLE 2. EFFECT OF SPLENIC IMPLANTS OF TESTOSTERONE PROPIONATE ON THE WEIGHTS OF SEMINAL VESICLES AND PROSTATE IN CASTRATED RATS ON VITAMIN B-COMPLEX-FREE AND YEAST-SUPPLEMENTED DIETS. SECOND SERIES, LONG-EVANS STRAIN[1]

Rat No.	Interval Between Castration and Implantation days	Body Wt. at Beginning of Diet gm.	Days on Diet	Body Wt. at End of Experiment gm.	Average Amt. Testosterone Propionate Absorbed per Day mg.	Seminal Vesicles Weight mg.	Prostate Weight mg.
Group 1, pellet subcutaneously, B-free diet+25% yeast							
1	20	225	34	325	0.171	586	921
2	20	190	34	270		330	758
3	20	190	34	255	0.188	461	1009
4	20	190	34	305		483	914
						mean 465	901
Group 2, pellet subcutaneously, B-free diet							
5	20	180	29	90	0.169	411	622
6	20	215	34	120		450	743
7	20	200	34	130	0.182	528	959
8	20	185	31	98	0.155	337	455
						mean 432	695
Group 3, pellet in spleen, B-free diet+25% yeast							
9	20	225	34	280	0.156	29	25
10	20	225	34	300	0.141	19	28
11	20	220	34	335	0.159	16	24
12	20	165	34	250	0.174	19	30
						mean 20	27
Group 4, pellet in spleen, B-free diet							
13	20	170	34	110	0.179	15	46
14	20	185	34	110	0.165	14	26
15	20	190	34	125	0.174	15	35
16	20	215	34	152	0.171	23	21
						mean 17	32
Group 5, no pellet, B-free diet							
17		145	34	100		15	25
18		145	31	90		16	24
19		290	38	160		28	27
20		315	38	140		28	—
21		270	38	155		26	29
22		270	38	190		20	25
23		350	38	170		27	—
24		340	38	155		34	28
						mean 24	26

[1] All animals in this series were castrated at the same age, when the body weight was approximately 100 gm. Except in *group 5*, the experimental diet was begun on the day the pellet was implanted.

of normal, unoperated animals was given a B-free diet. At the end of the experiment, each animal was killed and the contents of the peritoneal cavity were carefully examined. Three animals were found to have vascularized adhesions which might have permitted blood from the spleen to by-pass the liver; these were excluded. In the re-

maining animals, the seminal vesicles were removed, carefully pressed free of their contents and weighed immediately. The pellets were removed, dried *in vacuo* over calcium chloride and weighed on a torsion micro-balance.

In the second series of experiments (table 2) male rats of the Long-Evans strain were castrated at an earlier age than those of the first series, at an average weight of about 100 gm. When the animals were of weights ranging from 165 to 225 gm., pellets of testosterone propionate weighing from 10.6 to 12.5 mg., were implanted either subcutaneously or into the spleen. Five groups of animals were studied. These were treated as follows: *a*) Pellet subcutaneously, vitamin B complex-free diet plus 25 per cent brewers' yeast.[3] *b*) Pellet subcutaneously, vitamin *B* complex-free diet. *c*) Pellet in the spleen, vitamin B complex-free diet plus 25 per cent brewers' yeast. *d*) Pellet in the spleen, vitamin B complex-free diet. *e*) No pellet, vitamin B complex-free diet.

In this series, at the end of the experiment both seminal vesicles (emptied of their contents) and prostate were weighed on a chainomatic balance with a magnetic damper. The pellets were removed, dried and similarly weighed.

RESULTS AND DISCUSSION

It is apparent from the results obtained that deficiency of the vitamin B complex had no significant effect on inactivation of testosterone propionate in the liver. At most, in the first series of experiments, a slight difference may be noted between the average weight of the seminal vesicles of animals with splenic implants on a B-free diet and those of animals with splenic implants and a normal diet or of castrated animals (without implants) on a B-free diet. But when one considers the comparatively enormous change in the weights of the seminal vesicles produced by subcutaneous implants of testosterone propionate (table 2) this difference can hardly be considered physiologically significant. In the second series, which was better controlled, even this slight difference is not apparent.

These results are in agreement with the observations of Glass, Edmondson and Soll (9) on the excretion of free and combined estrogens and androgens in the urine of male patients with cirrhosis of the liver, all of whom had testicular atrophy, gynecomastia or both. They found that while all, or almost all, of the estrogen was excreted in active, uncombined form in these patients, free androgen was not found. Not only was there an absolute increase in the excretion of estrogen in these cases over the values for normal men, but in most of the cases there was also a diminution in the excretion of androgen. The latter may have been due, at least in part, to depression of the gonadotropic function of the pituitary by the excess of free estrogen.[3a]

As experimental cirrhosis of the liver is now known, from the studies of György and Goldblatt (10–12) and of others (13–17), to result from nutritional deficiency and as both clinical and experimental cirrhoses respond to nutritional therapy (18–22), it appears that the clinical findings of Glass and his associates and our experimental results have a common basis. It seems probable that the inactivation of androgens in the liver is accomplished by a mechanism different from that required for the estrogens.

[3] Anheuser Busch strain 'G' containing approximately 0.15 mg. of thiamin and 0.07 mg. of rivoflavin per gm. Twenty-five per cent of this preparation added to the vitamin B-free diet has been found adequate to restore the hepatic estrogen-inactivating mechanism in from 2 to 6 days.

[3a] Diminution in secretion of androgen in animals with intact testes on a B-free diet is indicated by the low weights of the seminal vesicles in *group 6*, table 1. The testicles of these animals were also markedly atrophic. The results obtained with *group 2*, table 2, show that the seminal vesicles (and the prostate) do not lose their ability to respond to androgen in deficiency of the B complex. That the atrophy of the testes which occurs in B complex deprivation is not solely due to the accompanying inanition is indicated in studies on pantothenic acid by Lepkovsky and Krause (cited by S. Lepkovsky in Biological Actions of the Vitamins, edited by E. A. Evans, Jr., Univ. of Chicago Press, 1942).

Failure of the liver to inactivate estrogen in a deficiency of the vitamin B complex while this organ continues to inactivate androgen must seriously disturb the estrogen-androgen equilibrium.[4] One possible consequence of such an alteration is indicated by the work of Lipschütz and his collaborators (23–25). They have shown that subserous fibroids can be produced by estrogen not only in the uterus but also in other abdominal organs and in the abdominal wall. Fibroids thus produced can be prevented by the simultaneous administration of testosterone (or of other steroids having the androstene nucleus). An absolute rise in body estrogen, not accompanied by a physiologically comparable rise in androgen, would favor the production of such tumors.

Some of the other possible consequences of the rise in body estrogen resulting from failure of its inactivation in the liver, have been pointed out in our previous papers (2, 3) and two of the syndromes there indicated (menorrhagia and chronic cystic mastitis) as well as a third (deficient spermatogenesis with sterility in the male)[5] have already been treated successfully with vitamin B complex and will be the subject of future reports.

Independent support of the thesis that vitamin B deficiency results in a protracted rise in body estrogen has recently been provided by Bean (26). He has made the interesting observation that the cutaneous vascular spiders and palmar erythema formerly associated mainly with cirrhosis of the liver, occur also in nutritional deficiency and in pregnancy. Bean has shown that such lesions can be intensified and new ones made to appear by the administration of estrogen and that these lesions regress on withdrawal of the estrogen. Perera (27) has also recently reported the appearance of palmar erythema in nutritional deficiency; he noted regression of this lesion in two cases of hepatic cirrhosis under dietary and vitamin therapy.

Not the least important of the possible consequences of the failure of the liver to inactivate estrogen in deficiency of the vitamin B complex is the establishment of what we believe to be a vicious cycle. For not only does vitamin B deficiency cause impairment of the estrogen-inactivating mechanism, but as Ashworth and Sutton (28) have shown, estrogen itself may cause vitamin B deficiency. The observations of Ashworth and Sutton in patients are in agreement with unpublished observations by one of us (M.S.B) on animals.[6] Female rats having subcutaneous implants of diethylstilbestrol or receiving injections of large doses of this substance, when placed on a vitamin B-complex-free diet showed signs of nutritional deficiency much earlier and lost weight more rapidly than animals not treated with estrogen. Graded supplements of brewers' yeast in the diet of the rats treated with estrogen delayed the appearance of the cutaneous lesions characteristic of B deficiency and diminished the loss of weight.

It seems probable that estrogens can produce vitamin B deficiency owing to actual utilization of factors of the B complex during the inactivating process, resulting in what Rhoads has called a 'conditioned' deficiency.

SUMMARY

Vitamin B-complex deficiency does not significantly impair the inactivation of testosterone propionate in the liver of the male rat. This is in contrast to the results previously obtained with estrogens, the inactivation of which is strikingly impaired

[4] It should be pointed out that even if the inactivation of androgens by the liver were impaired to the same degree, on a weight for weight basis, as that of the estrogens, the equilibrium would still be shifted toward the estrogens, owing to the very much higher physiologic activity of the latter per unit weight.

[5] M. S. B. in collaboration with Dr. Henry C. Falk.

[6] In collaboration with Dr. Henry Brody.

in vitamin B deficiency. Together with an absolute rise in the level of body estrogen which thus results, a serious alteration must occur in the estrogen-androgen equilibrium. Possible clinical consequences of these disturbances are discussed.

REFERENCES

1. Biskind, M. S., and G. R. Biskind: *Science* 94: 462. 1941.
2. Biskind, M. S., and M. C. Shelesnyak: *Endocrinology* 30: 819. 1942.
3. Biskind, M. S., and G. R. Biskind: *Endocrinology* 31: 109. 1942.
4. Biskind, G. R., and J. Mark: *Bull. Johns Hopkins Hosp.* 65: 212. 1939.
5. Biskind, G. R.: *Proc. Soc. Exper. Biol. & Med.* 43: 259. 1940.
6. Biskind, G. R.: Medico-Surgical Tributes to Harold Brunn, University of California Press, 1942. p. 41.
7. Burrill, M. W., and R. R. Greene: *Endocrinology* 31: 73. 1942.
8. Selye, H.: *J. Pharmacol. & Exper. Therap.* 71: 236, 1941.
9. Glass, S. J., H. A. Edmondson and S. N. Soll: *Endocrinology* 27: 749. 1940.
10. György, P., and H. Goldblatt: *J. Exper. Med.* 70: 185. 1939.
11. György, P., and H. Goldblatt: *J. Exper. Med.* 72: 1. 1940.
12. György, P., and H. Goldblatt: *J. Exper. Med.* 75: 355. 1942.
13. Rich, A. R., and J. D. Hamilton: *Bull. Johns Hopkins Hosp.* 66: 185. 1940.
14. Lillie, R. D., F. S. Daft and W. H. Sebrell, Jr.: *Pub. Health Rep.* 56: 1255. 1941.
15. Daft, F. S., W. H. Sebrell and R. D. Lillie: *Proc. Soc. Exper. Biol. & Med.* 48: 228. 1941.
16. Earle, D. P., Jr., and J. Victor: *J. Exper. Med.* 73: 161. 1941.
17. Blumberg, H., and E. V. McCollum: *Science* 93: 598. 1941.
18. Patek, A. J., Jr.: *Proc. Soc. Exper. Biol. & Med.* 37: 329. 1937.
19. Patek, A. J., Jr., and L. Post: *J. Clin. Investigation* 20: 481. 1941.
20. Lowry, J. V., F. S. Daft and others: *Pub. Health Rep.* 56: 2216. 1941.
21. Broun, G. O., and R. O. Muether: *J. Am. Med. Assoc.* 118: 1403. 1942.
22. Lillie, R. D., L. L. Ashburn and others: *Pub. Health Rep.* 57: 502. 1942.
23. Lipschütz, A., and L. Vargas, Jr.: *Endocrinology* 28: 669. 1941.
24. Lipschütz, A., L. Vargas, Jr., and C. Nuñez: *Proc. Soc. Exper. Biol. & Med.* 48: 271. 1941.
25. Lipschütz, A., and J. Zañartu: *Endocrinology* 31: 192. 1942.
26. Bean, W. B.: *Am. J. M. Sc.* 204: 251. 1942.
27. Perera, G. A.: *J. Am. Med. Assoc.* 119: 1417. 1942.
28. Ashworth, J., and D. C. Sutton: *Arch. Int. Med.* 69: 15. 1942.

EFFECT OF HYPOPHYSECTOMY ON THE CONCENTRATION OF ASCORBIC ACID IN THE ADRENALS OF THE RAT[1]

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SINCE THE ISOLATION of ascorbic acid from the adrenal cortex by Szent-Györgyi, special importance has been attached to its high concentration in this organ. Numerous investigations have been made in an effort to determine the significance of its occurrence there in relation to adrenal-cortical function. In patients with Addison's disease, and in animals with adrenal insufficiency, the administration of vitamin C, either alone or combined with adreno-cortical extracts, has failed to show a therapeutic effect. Reports in the literature seem to indicate that the concentration of ascorbic acid in the adrenals may be an index of its activity. Leblond (1) has observed that the adrenal cortex of hypophysectomized rats contains only 45 per cent of the normal amount of ascorbic acid 3 months after operation, and similar values were reported by Bowman, Morris and Stacy (2). The low concentration of ascorbic acid after hypophysectomy may be an index of the decreased metabolic activity in the adrenal cortex. Therefore, in the present study the change in the content of ascorbic acid of the adrenals was compared with the change in their weight and function. The rate of regression in weight of the adrenal cortex following hypophysectomy has been reported in an earlier communication (3) as has been the decrease in its function (4) when measured by the resistance of hypophysectomized rats to low environmental temperatures. Considerable regression in the weight of the adrenals has been found within the first week after the removal of the hypophysis, starting 2 days after operation, while the impaired adreno-cortical function became apparent as aoon as the hypophysis had been removed. In the experiments reported below on suprarenal glands of rats we have studied the rate of regression in weight, as well as the rate of decrease of their content of ascorbic acid.

METHODS

Male rats, 21 to 40 days old and weighing from 35 to 100 gm. were hypophysectomized in groups of 15 to 20 and were then killed at various intervals after the operation. Forty-nine of the animals were killed with chloroform and only the adrenals dissected for the determination of ascorbic acid. Twelve normal animals served as controls. Eithteen hypophysectomized rats were killed under ether anesthesia by exsanguination. In those animals the concentration of ascorbic acid was determined in the blood serum, the liver, adrenals, kidneys, and testes. Similar determinations were made in 10 normal rats, maintained on the usual diet consisting of purina fox chow,

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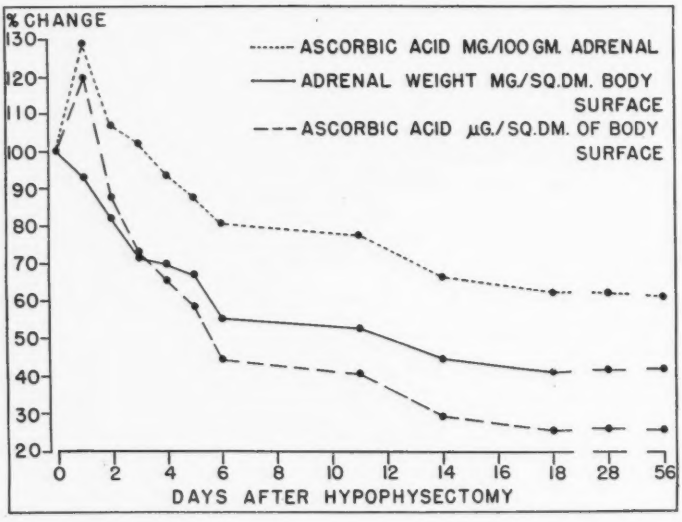
% CHANGE
ASCORBIC ACID MG./100 GM. ADRENAL
ADRENAL WEIGHT MG./SQ.DM. BODY SURFACE
ASCORBIC ACID µG./SQ.DM. OF BODY SURFACE
DAYS AFTER HYPOPHYSECTOMY

Fig. 1. Effect of hypophysectomy on weight and ascorbic acid concentration of the adrenals of male rats.

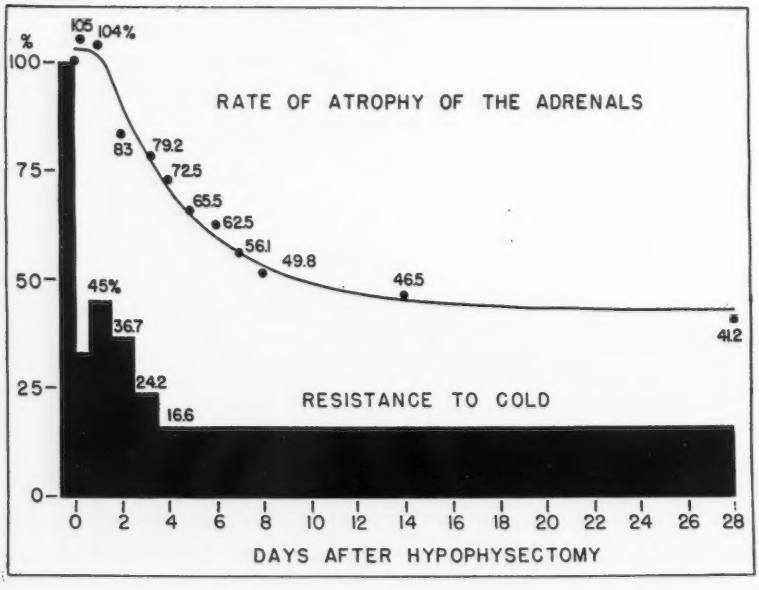

Fig. 2. Rate of atrophy of the adrenal is represented by a curve expressing a logistic function. The black space indicates the number of hours the body temperature could be maintained above 30° C. in an environment of 0° C., calculated as a percentage of the normal value.

and in 5 normal rats which had been starved for 6 days. The adrenal weight and content of ascorbic acid were determined in 5 hypophysectomized and in 4 hypophysectomized-thyroidectomized rats which had been injected with a pituitary extract with corticotropic activity. The ascorbic acid of the adrenals was also determined in 3 normal female and 3 hypophysectomized female rats.

The organs, or parts of them which were dissected rapidly on moist absorbent

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON WEIGHT AND ASCORBIC ACID CONCENTRATION OF THE ADRENALS

Time in Days After Hypophysectomy	No. of Animals	Adrenal Weight[1]	Percentage of Normal	Ascorbic Acid			
				mg./100 gm. of adrenal	%	Micrograms per Adrenal[1]	%
Normal Animals	21	8.9 (7.9–9.8)	100	351 (262–434)	100	31.2 (41.5–22.0)	100
1	3	8.3 (7.2–9.0)	93.0	454 (420–485)	129	37.6 (33.2–43.8)	120
2	19	7.3 (6.2–8.9)	82.0	377 (315–458)	107	27.4 (21.0–33.7)	87.6
3	13	6.4 (5.3–8.4)	71.5	359 (317–431)	102	22.8 (18.4–30.0)	73.0
4	7	6.2 (5.5–7.1)	70.0	329 (260–428)	93.5	20.4 (14.2–30.2)	65.5
5	2	6.0 (5.7–6.2)	67.0	307 (269–346)	87.5	18.3 (15.3–21.4)	58.6
6	2	4.9 (4.8–5.1)	55.2	283 (276–291)	80.6	13.9 (13.9–13.9)	44.5
11	2	4.7 (4.0–5.4)	52.6	272 (233–312)	77.5	13.0 (9.2–16.8)	41.2
14	2	4.0 (3.9–4.1)	44.6	233 (202–264)	66.4	9.3 (7.9–10.7)	29.6
18	4	3.7 (3.1–4.3)	41.2	218 (199–235)	62.2	8.0 (6.7–9.0)	25.6
28	4	3.7 (3.2–4.1)	41.8	219 (165–252)	62.4	8.1 (6.2–12.6)	26.0
56	7	3.8 (3.2–4.5)	42.0	216 (163–257)	61.5	8.1 (6.4–10.1)	25.9

[1] The adrenal weight was expressed in mg. per square decimeter of body surface.

paper, were weighed on a torsion balance and extracted with 3 per cent metaphosphoric acid. Blood was centrifuged immediatey after withdrawal from the heart, and the serum was used for the determination of ascorbic acid which was made with a photoelectric colorimeter according to the method of Mindlin and Butler (5). For the determination of ascorbic acid in tissues the method of Bessey (6) was applied to eliminate the errors arising from the turbidity and color of the fluids. A hypophyseal extract of known corticotropic potency, similar to those employed in previous experiments on adrenal function (4), was used.

RESULTS

Figure 1 shows the rate of regression in adrenal weight and the change in the concentration of ascorbic acid expressed in percentage of the normal values. The absolute figures are shown in table 1. It is apparent that the adrenal weight of hypophysectomized rats began to decrease within the first two days after operation. The ascorbic acid concentration, on the other hand, showed an increase during this period, and then dropped below its normal level after 3 days. Six days after hypophysectomy the adrenal weight had decreased by 45 per cent while the ascorbic acid concentration had decreased by only 20 per cent. Fourteen days after operation the weight had decreased 55 per cent, the ascorbic acid content 34 per cent. Fifty-six days after operation, these decreases were 58 and 38.5 per cent, respectively.

The results of the determination of ascorbic acid in various organs and in blood

TABLE 2. EFFECT OF HYPOPHYSECTOMY AND OF STARVATION ON THE CONCENTRATION OF ASCORBIC ACID IN VARIOUS TISSUES.

Time After Hypophysectomy	Number of Animals	Ascorbic Acid, mg. per 100 gm.				
		Adrenal	Testis	Liver	Kidney	Serum
Normal Animals	9	360 (434–272)	42.5 (62.5–28.7)	28.6 (34.7–21.9)	15.6 (22.1–11.6)	1.08 (1.47–0.75)
3 days	3	397 (475–340)	44.0 (47.1–42.0)	26.2 (27.9–24.2)	19.8 (22.1–18.0)	0.95 (1.03–0.88)
2 weeks	2	233 (264–202)	28.3 (28.7–28.0)	17.6 (19.8–15.5)	9.5 (9.66–9.30)	0.65 (0.86–0.45)
4 weeks	4	219 (252–165)	25.4 (29.5–22.0)	16.9 (20.4–12.3)	12.6 (14.1–10.5)	0.80 (0.98–0.59)
8 weeks	7	216 (257–163)	24.8 (28.7–15.2)	16.9 (22.4–11.4)	11.7 (16.5–8.08)	0.66 (0.86–0.44)
% of normal		60%	58.4%	59%	75%	61%
Unoperated animals starved for 6 days	5	320 (358–241)	33.6 (39.1–29.6)	17.1 (20.9–13.0)	8.6 (12.5–6.8)	0.92 (1.22–0.65)
% of normal		89%	79%	60%	55%	85%

serum of hypophysectomized and starved animals are shown in table 2. It can be seen that at the end of the eighth week after hypophysectomy the degree of reduction in the ascorbic acid of the testis, liver, kidney, and blood serum was similar to that observed in the adrenal. For the liver and kidney only, did starvation cause a decrease of ascorbic acid comparable to that observed after hypophysectomy; the reduction in adrenal, testis, and blood serum was less.

The data in table 3 show the changes in the ascorbic acid content of the adrenals of female rats 10 days after hypophysectomy. They are similar to those observed in males. In this table, also, is shown the effect of an injected pituitary extract and of incomplete hypophysectomy on the adrenals. In both instances the ascorbic acid concentration was increased. Table 3 likewise contains the results of determinations of ascorbic acid in hypophysectomized-thyroidectomized rats. These animals exhibited changes comparable to those shown by hypophysectomized rats in which the thyroid was left intact.

TABLE 3. A. EFFECT OF HYPOPHYSECTOMY ON THE WEIGHT AND ASCORBIC ACID CONCENTRATION OF THE ADRENALS OF FEMALE RATS. CHANGES OBSERVED 10 DAYS AFTER HYPOPHYSECTOMY. B. EFFECT OF A PITUITARY EXTRACT AND OF INCOMPLETE HYPOPHYSECTOMY ON THE ASCORBIC ACID CONCENTRATION OF THE ADRENALS OF MALES, AS DETERMINED 18 DAYS AFTER OPERATION. C. HYPOPHYSECTOMIZED-THYROIDECTOMIZED MALE RATS UNTREATED AND INJECTED WITH PITUITARY EXTRACT. THE ANIMALS WERE KILLED 14 DAYS AFTER OPERATION

	Body Weight, gm.		Adrenal Weight		Ascorbic Acid	
	Initial	Final	Total mg.	Mg. per square decimeter of body surface	Mg. per 100 grams of adrenal	Micrograms per amount adrenal[1]
A. 3 Normal females	56	93	17.7	9.32	298 (328–280)	27.8 (32.0–24.8)
3 Hypophysectomized females	58	63	7.5	5.19 (55.6%)	199 (218–171) (66.7%)	10.2 (11.0–9.8) (36.6%)
B. 4 Hypophysectomized, untreated	119	110	8.1	3.66	218	7.9
5 Hypophysectomized, untreated for 13 days and then injected for 5 days with 20 mg. equivalent daily	119	122	11.2	5.04	277	13.9
2 Incompletely hypophysectomized, untreated	98	118	14.8	7.56	381	28.9
C. Hypophysectomized-thyroidectomized: 3 controls	88	73	5.6	3.05	175	5.3
4 Injected with 20 mg. equivalent daily for the last 7 days	88	87	10.5	5.70	223	12.7

[1] Calculated on the basis of adrenal weight per sq. decimeter of body surface.

DISCUSSION

Considering only the lowered values of ascorbic acid in the adrenals, as effected by hypophysectomy, and as shown in tables 1 and 3, one could easily be led to believe that hypophysectomy decreases the ascorbic acid of the adrenals in a specific way. From table 2, however, it is apparent that the amount of ascorbic acid in the testis, liver, kidney, and blood serum is also lowered to a similar extent, the reduction being calculated as a percentage of the initial value. Starvation, on the other hand, reduces the concentration of ascorbic acid in the liver and kidney to a greater degree than in the adrenals, testis or blood serum. One must, therefore, assume that the change in the concentration of ascorbic acid which takes place in the adrenals of hypophysectomized rats is not specific for this organ. Furthermore, this reduction cannot be considered as indicative of the approaching atrophy, since it is preceded both by the weight decrease of the adrenals and by their impaired function. Figure 1 shows for comparison the rate of decrease in adrenal weight and in ascorbic acid concentration. Figure 2 correlates the decrease in weight and decrease in function of the adrenal glands; figure 2 is based on experimental data reported previously (3), in which the adrenal function of hypophysectomized rats was measured by their resistance upon exposure to an environmental temperature of 0°C. From figure 2 it is apparent that the resistance

decreases rapidly with the removal of the hypophysis while the atrophy occurs relatively more slowly. As adrenal function is impaired several days prior to a change in the concentration of ascorbic acid, it is apparent that changes in the level of this substance are of no value as an index of adrenal cortical activity.

SUMMARY

The weight of the adrenals and the concentration of ascorbic acid in the adrenals of the rat were determined 1 to 56 days after hypophysectomy. A gradual decrease was observed, which, however, did not seem to be specific for this organ, as the concentration of ascorbic acid in the testis, liver, kidney, and blood serum showed assimilar decrease. Starvation of normal rats for 6 days reduced the ascorbic acid in the liver and kidney to a greater degree than in the adrenal, testis or blood serum. Analysis of the adrenals of normal and hypophysectomized female rats showed values for ascorbic acid which were similar to those in males. The concentration of ascorbic acid in the adrenals of hypophysectomized rats was increased by treatment with suitable pituitary extracts. In instances of incomplete hypophysectomy the values were found to be high. Removal of the thyroid, in addition to hypophysectomy, in rats did not alter the results observed upon treatment in hypophysectomized animals.

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REFERENCES

1. LEBLOND, C. P., AND A. CHAMORRO: *Compt. rend. Soc. de biol.* 133: 71. 1940.
2. BOWMAN, D. E., L. E. MORRIS AND J. R. STACY: *Proc. Soc. Exper. Biol. & Med.* 45: 784. 1940.
3. TYSLOWITZ, R., AND E. B. ASTWOOD, *Federation Proceedings.* 1: 86. 1942.
4. TYSLOWITZ, R., AND E. B. ASTWOOD: *Am. J. Physiol.*, 136: 22. 1942.
5. MINDLIN, R. L., AND A. M. BUTLER: *J. Biol. Chem.* 122: 673. 1937.
6. BESSEY, O. A.: *J. Biol. Chem.* 126: 771. 1938.

PROLONGATION OF SEX HORMONE EFFECTS BY ADSORPTION ON POWDERED CARBON[1]

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SPEED of absorption is important in hormone effectiveness. A given quantity of hormone exerts a different effect depending upon whether it is administered in a series of injections (1–4), or whether it is in rapidly or slowly absorbed vehicles (5–7). Butenandt (8) as well as Miescher (9) have shown that the esters of steroid hormones produce a more prolonged effect than their non-esterified counterparts because the former are absorbed more slowly. This holds true also of treatment with hormone crystals (10).

We have attempted to prolong the period of absorption by the administration of hormones on various adsorbates. It was assumed that the adsorbed hormone would be washed out slowly by the organism and thus exert a prolonged action.

The effectiveness of the following adsorbents were investigated: animal and plant carbon, kaolin, kieselgur, Fuller's earth, calcium carbonate, benzoic acid, flexible collodiun and Lloyd's reagent. Of all of these adsorbents, only animal and plant carbon powder proved definitely effective in prolonging the hormone effect.

EXPERIMENTAL PROCEDURE AND RESULTS

Estrogens. Experiments in this group dealt with the prolongation of the effect of estrone,[2] estradiol benzoate[2] and diethylstilbestrol[2] when adsorbed on powdered carbon. Each 100 I.U. (0.01 mg.) of estrogenic substance was dissolved in 2 cc. of 96 per cent alcohol and 4 mg. of carbon powder was added, this quantity of carbon having been proven optimal in prolonging the hormone effect. The alcohol was then evaporated during continuous shaking of the hormone-carbon mixture. The resulting carbon-hormone powder was implanted subcutaneously into adult spayed female mice. The occurrence of estrus was observed by the making of vaginal smears every 12 hours. Some animals received the carbon-hormone powder in the form of tablets placed subcutaneously. Tablets of this kind were prepared by mixing some drops of a concentrated solution of gum arabic with the above described hormone-carbon powder and drying at 70° C.

In control experiments, spayed mice were given one subcutaneous injection with 0.01 mg. of estrogenic substance dissolved in 0.5 cc. N/100 NaOH or 0.5 cc. of olive oil. Crystals were administered suspended in a 10 per cent solution of gum arabic, so that 0.5 cc. of the suspension contained 0.01 mg. of finely powdered crystals of estrogenic substance.

A total of 120 spayed mice were used for the experiments, the data of which are recorded in table 1.

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[2] We are indebted to Dr. Brent, Roche-Organon, Inc., Nutley, N. J. for the supply of estrone and estradiol benzoate and to 'Teva,' Middle East Pharmaceutical and Chemical Works Co., Ltd., Jerusalem, for the supply of diethylstilbestrol.

These results demonstrate that the estrus, produced by estrone adsorbed on carbon powder, is of longer duration than that produced by estrone dissolved either in N/100 NaOH, in oil, or administered in crystal form. One hundredth of a mg. of estrone adsorbed on carbon powder sustained estrus for 16 days, while the same amount of estrone dissolved in N/100 NaOH or olive oil elicited estrus of only 1 day in duration. Estrone crystals produced estrus of only 5 days' duration. The adsorption on carbon prolongs the action of estrone 16-fold as compared with the usual solution of estrone in oil and 3-fold, as compared with crystals. Estrone adsorbed on carbon and implanted in tablet form gave a somewhat shorter period of estrus, lasting only 12 days.

The action of estradiol benzoate is not prolonged in the same way by adsorption on carbon. One-hundredth of a mg. of this substance in oil produced an estrus which

TABLE 1. DURATION OF ESTRUS IN MICE FOLLOWING ADMINISTRATION OF ESTROGENIC SUBSTANCE IN VARIOUS FORMS

Estrogen Used	Quantity, mg.	Number of Animals	Average Duration of Estrus, days
Estrone in N/100 NaOH	0.01	20	1
Estrone in oil	0.01	5	1
Estrone crystals	0.01	10	5
Estrone adsorbed on carbon powder	0.01	20	16
Estrone adsorbed on carbon as tablet	0.01	5	12
Estradiol benzoate in oil	0.01	15	18
Estradiol benzoate crystals	0.01	10	31
Estradiol benzoate adsorbed on carbon powder	0.01	10	30
Estradiol benzoate on carbon as tablet	0.01	5	35
Diethylstilbestrol in N/100 NaOH	0.01	10	1
Diethylstilbestrol, adsorbed on carbon powder	0.01	10	1

lasted 18 days, while the same amount adsorbed on carbon powder induced an estrus lasting 30 days. One-hundredth of a mg. of estradiol benzoate crystals, however, induced estrus of the same duration (31 days). Estradiol benzoate adsorbed on carbon and implanted in a tablet form sustained a somewhat longer estrus (35 days).

Adsorption of diethylstilbestrol on carbon does not prolong its action.

Progesterone. The technique employed in the study of progesterone was the same as described for estrone. Each mg. of progesterone was adsorbed on 10 mg. of carbon powder. The progesterone was tested as follows. Female rabbits weighing 1000 gm. were treated daily for 8 days with 20 I.U. of estrone, following which progesterone adsorbed on carbon powder was implanted subcutaneously. Five days after implantation the animals were killed and their uteri examined histologically. The experiments were conducted on 10 animals. They showed that 3 mg. of progesterone adsorbed on 30 mg. of carbon were needed to produce a complete progestional transformation of the uterine mucosa, while 40 mg. of progesterone dissolved in 2 cc. of olive oil was not sufficient to produce the same effect when given in one injection.[3] This demonstrates that the action of progesterone action is prolonged at least 14 times by adsorption on carbon.

Testosterone. The technique employed with testosterone was the same as for the foregoing hormones. Fifty mg. of carbon powder was added to 5 mg. of testosterone

[3] When administered in 5 injections, 1 mg. of progesterone is sufficient to produce complete progestional transformation of the uterine mucosa.

dissolved in 2 cc. of ethyl alcohol. The alcohol was evaporated during continuous shaking of the mixture. The resulting powder was then implanted subcutaneously into male rats, which were castrated 4 weeks before the beginning of the experiment when they weighed 60 gm. Ten days after testosterone implantation the animals were killed, the sex organs fixed in 4 per cent (1:10) formalin, and weighed 24 hours later. The same procedure was followed in control experiments using 5 mg. of testosterone dissolved in 1 cc. of olive oil and administered in one injection, or with 5 mg. of testosterone crystals implanted subcutaneously. The results of these experiments are shown in table 2.

The data in table 2 show that the weight of the individual sex glands and their total weight is about 5 times as high after administration of testosterone adsorbed on

TABLE 2. EFFICACY OF TESTOSTERONE IN VARIOUS FORMS

No. of Rats	Testosterone, mg.	Preparation Vehicle	Average Weight of Sex Glands, mg.		
			Seminal vesicles	Prostate	Total
5	5	Oil	25	52	312
5	5	Crystals	135	195	805
5	5	Adsorbed on carbon powder	295	345	1526

carbon than after administration of testosterone dissolved in oil. Following the administration of testosterone in crystal form the sex glands weighed only half as much as after administration of carbon-testosterone.

Chorionic gonadotropin and insulin. Attempts were made to prolong the action of chorionic gonadotropin and insulin. The experiments gave, however, negative results, probably because these hormones are eluted in the organism with relative rapidity. The effect of carbon-powder in prolonging hormone action seems to be limited to fat soluble hormones.

DISCUSSION

It seems clear that the action of certain steroid hormones is prolonged by adsorption on carbon. This prolongation of hormone effect does not hold true, however, for water-soluble hormones such as chorionic gonadotropin or insulin. The experiments show that the prolongation of hormone effect by carbon adsorption is most pronounced for estrone (16-fold). The action of estradiol benzoate is not significantly prolonged (2-fold) by the same treatment, since, being an ester, the absorption of this substance is slow in any case. Progesterone on carbon acts at least 15 times longer than when administered in oil solution in one injection. Testosterone on carbon is 5 times as effective as testosterone in oil administered in one injection and 2 times as effective as in crystal implantation. The action of diethylstilbestrol could not be prolonged by adsorption, this probably being due to a looser adsorption of this substance to the carbon powder.

SUMMARY

The action of the steroid hormones estrone, progesterone and testosterone in the organism is prolonged by adsorption on powdered carbon. This does not hold true, however, for diethylstilbestrol.

The activity of the water-soluble hormones, chorionic gonadotropin and insulin, is not influenced by similar adsorption.

I am indebted to Prof. B. Zondek for his kind guidance throughout this work.

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REFERENCES

1. LAQUEUR, E., AND S. E. DE JONGH: *J. Pharmacol. & Exper. Therap.* 36: 1. 1929.
2. MARRIAN, G. F., AND A. S. PARKES: *J. Physiol.* 67: 389. 1929.
3. ALLAN, H. F., E. C. DICKENS AND E. C. DODDS: *J. Physiol.* 68: 348. 1930.
4. BUTENANDT, A., AND E. ZIEGNER: *Ztschr. f. physiol. Chem.* 188: 1. 1930.
5. BURN, J. H.: *Quart. J. Pharm. & Pharmacol.* 2: 63. 1939.
6. BURN, J. H., AND G. K. ELPHIC: *Quart. J. Pharm. & Pharmacol.* 5: 192. 1932.
7. ZONDEK, B: Hormone d. Ovariums u. do. Hypophysenvorderlappens. 2nd ed. Julius Springer, Vienna, 1935. p. 125.
8. BUTENANDT, A: *Ztschr. f. physiol. Chem.* 191: 140. 1930.
9. MIESCHER, K., C. SCHOLZ AND E. TSCHOPP: *Biochem. J.* 32: 725. 1938.
10. DEANESLY, R., AND A. S. PARKES: *Proc. Roy. Soc. London, s. B.* 124: 279. 1937.

BEGINNING OF FUNCTION IN THE THYROID OF THE FETAL RAT

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THE DETERMINATION of the time of beginning of function in embryonic endocrine glands has long interested embryologists and physiologists, especially since the data accumulated so far attest to the fact that hormones are imporatnt morphogenetic agents in developing organisms as well as in adults. The spectacular participation of the thyroid in the metamorphosis of larval amphibia is an example of this. The postponement of cachectic symptoms in thyroidectomized pregnant mammals until delivery of full term fetuses provides the hint that fetal thyroids may contribute significantly to the physiologic economy of the mother.

In the present experiments radio-active iodine[1] was injected into rats in a known

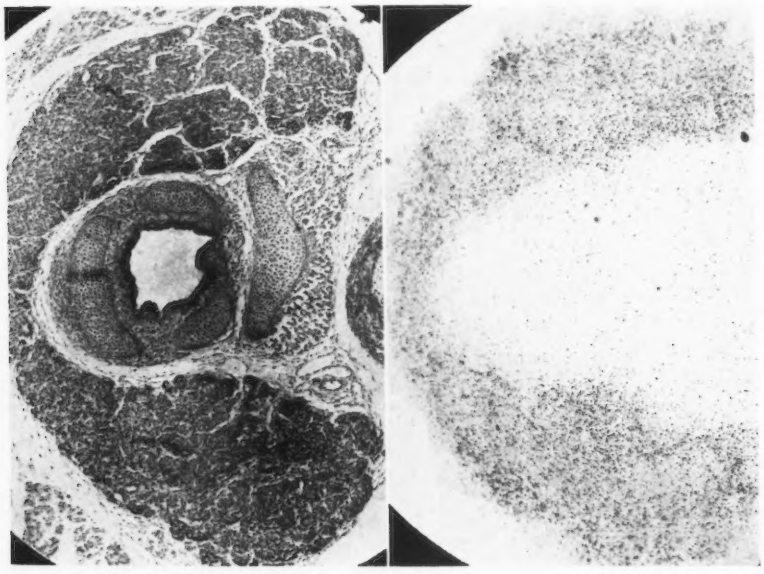

Fig. 1. HISTOLOGIC SECTION AND RADIO-AUTOGRAPH of thyroid of 19-day-old rat. Mother was injected with radio-active iodine.

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[1] We are indebted to the Radiation Laboratory of the University of California, and especially to Dr. J. G. Hamilton for the radio-active iodine used in these experiments.

stage of pregnancy, and the developing thyroids of the fetuses were examined by the radio-autograph method (1, 2) to determine the presence of accumulated iodine and its distribution. The iodine was given subcutaneously in a single injection of 1.0 cc. of physiologic saline solution containing 35 microcuries of radio activity. The mass of iodine was negligible, being less than 0.01 mg. (as NaI). The radio-activity in the preparation used was due almost exclusively to I^{131} an isotope of iodine having a half-life of 8 days. The pregnant female rats were killed 48 hours after injection and the

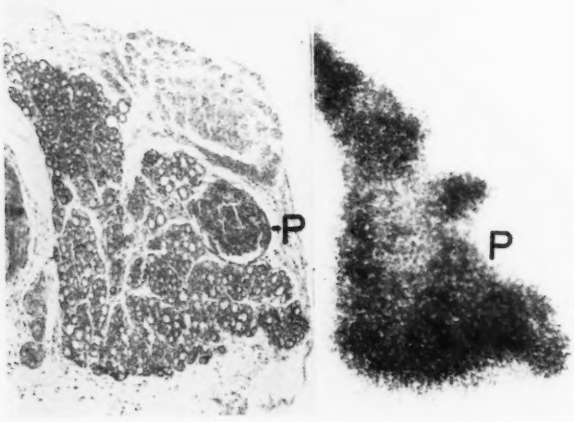

Fig. 2. HISTOLOGIC SECTION AND RADIO-AUTOGRAPH of thyroid of new-born (21-day-gestation) rat. Mother injected with radio-active iodine. Note absence of iodine in parathyroid gland (P).

fetuses were removed for fixation in formalin. Thyroids of 29 fetuses, of ages from 15 to 21 days (full term), at fixation, were examined. No iodine accumulation was found in fetuses less than 19 days old. Thyroids of 19-day-old fetuses showed, without exception, a relatively weak iodine accumulation (fig. 1). Therefore, it may be summarized that functional accumulation of iodine was initiated in the 18 to 19-day-old rat fetus. A considerably stronger storage of iodine was found in 21-day-old fetuses (fig. 2) which were fixed immediately at birth.

Definite histologic features can be correlated with the beginning of function in the thyroid. Thyroids in 19-day-old embryos, which were the youngest of those tested having the ability to store iodine, differ only slightly in structure from the thyroids of 18-day-old embryos which stored no iodine. The differentiation of follicles without lumina from the originally solid cords of cells is only slightly advanced in the 19-day-old fetus from the condition in the 18-day-old fetus. At the periphery of the 19-day thyroid a few small follicles with lumina are found. The deeper portions of the thyroid and the isthmus lag behind in the differentiation of definitive follicles. There is a recognizable, but slight, increase in vascularity between 18 and 19 days. In the 21-day thyroid an additional illustration of the relative advance in differentiation of peripheral follicles may be seen (fig. 2). It is interesting that physiologic differentiation parallels these features of morphologic development. Careful examination of the radio-autograph in figure 2 shows an area of light deposition of radio-iodine in its center which corresponds to the deeper tissue within the thyroid. It may be noted that in this inactive part of the 21-day thyroid, follicles with lumina have not yet differen-

tiated. In the more peripheral portions of the 21-day thyroid, on the other hand, numerous well-developed follicles are found and vascularity is also considerably increased. The corresponding areas in the radio autograph indicate a high degree of iodine storage. Another interesting feature, clearly demonstrated in figure 2, is the lack of storage of iodine in the parathyroid.

As indicated by the present data, thyroid function in the rat does not begin until 90 per cent of intra-uterine life has been completed. This may be contrasted with the condition found with the same technique in a free-living embryo, the frog tadpole. Here thyroid function begins soon after completion of the embryonic tail-bud stage and hatching from the egg (2). Beginning of function in the tadpole thyroid at such a relatively early stage of development may be correlated with the disappearance of stored yolk and beginning of independent feeding. Iodine storage in the endostyle which contains the anatomical anlage of the thyroid of larval lampreys (*Entosphenus lamottenii*) begins early in the first of the 5 years of larval life (3). The possibility may be suggested that a functional thyroid or thyroid-like tissue is a necessary element in the physiologic economy of a free-living developing animal.

SUMMARY

Functional ability to store iodine begins in the 18 to 19-day-old rat embryo. This may be correlated with the first differentiation of follicles with lumina. Follicular differentiation begins with the peripheral portions of the thyroid and gradually extends into the deeper portions.

REFERENCES

1. Hamilton, J. G., M. H. Soley and K. B. Eichorn: *Univ. of California Publ., Pharmacol.* 1: 339. 1940.
2. Gorbman, A., and H. M. Evans: *Proc. Soc. Exper. Biol. & Med.* 47: 103. 1941.
3. Gorbman, A., and C. W. Creaser: *J. Exper. Zoöl.* 89: 391. 1942.

NOTES AND COMMENTS

PRODUCTION OF TESTIS ATROPHY BY STEROIDS

It has been shown repeatedly that certain steroids, especially the estrane derivatives with folliculoid activity, as well as some of the testoid and cortical compounds, produce marked testis atrophy in the intact rat. This atrophy is mainly due to degeneration of the tubular elements and to a lesser extent is caused by involution of the Leydig cells. Testosterone exhibits a rather peculiar effect inasmuch as small doses cause more pronounced atrophy than large doses.[1] It has been emphasized that some folliculoid activity may be detected by special tests in all hormonally active steroids so far examined. Apart from the estrane derivatives, which of course are most active in this respect, testosterone and methyl testosterone proved to possess the highest folliculoid potency.[2] It has also been reported[3] that the testis atrophy caused by estradiol may be prevented by testosterone since the anti-spermatogenic effect of the former is counteracted by the spermatogenic action of the latter. However, comparatively large doses of testosterone are required to obtain this effect. Δ^5-Androstene-3(β),17(α)-diol, another testoid compound with marked spermatogenic activity, also antagonizes the testis atrophy caused by folliculoids, but when given in small amounts by itself it causes no testis atrophy comparable to that elicited by low doses of testosterone.[2] We tentatively suggested the theory that the inability of this androstenediol to cause testis atrophy at any dosage level is due to the fact that, judged by all available tests, it is much less folliculoid in its effects than testosterone. It was assumed that in the case of testosterone the anti-spermatogenic effect of its folliculoid action prevails over the spermatogenic potency of the compound at low, but not at high dosage levels. In the case of the androstenediol, however, the spermatogenic effect prevails at all dosage levels over the comparatively low folliculoid potency.

A recent experiment strongly supports the above concept. Sixteen male albino rats weighing 85 to 97 gm. (average 89 gm.) were divided into 2 groups. Eight animals were given 2 daily subcutaneous injections of 0.1 cc. of peanut oil containing 2.5 μg. of α-estradiol and 0.5 mg. of Δ^5-androstene-3(β),17(α)-diol (m.p. 182–183°C.). The remaining 8 rats received 25 μg. of estradiol and 5 mg. of androstenediol bidaily administered in the same manner and in the same amount of solvent. Thus the total daily dose was 5 μg. of estradiol and 1 mg. of androstenediol in the first group and 10 times this amount of the same steroid mixture in the second group. Treatment was continued for 14 days and the testes were weighed after fixation in Susa mixture following the autopsy which took place 24 hours after the last injection. It was found that in the group receiving the low dosage the average testis weight was 0.980 gm. (range: 0.758–1.105 gm.), while in the high dosage group it was 1.303 gm. (range: 1.167–1.756 gm.).

This experiment indicates that this androstenediol, which in itself causes no testis atrophy at any dosage level, has the property of eliciting pronounced testicular damage if it is administered in combination with a given percentage of estradiol. In common with testosterone, more pronounced damage is produced by the low dosage than the high. It may be said, therefore, that by suitable combination of two steroids, none of which acting alone possesses this

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[1] Selye, H.: *Proc. Soc. Exper. Biol. & Med.* 46: 142. 1941.

[2] Selye, H., and S. Albert: *Am. J. M. Sc.* In press.

[3] Selye, H.: *Canad. M. A. J.* 42: 113. 1940.

particular action of testosterone in producing atrophy of the testis, one may accomplish what might be termed the 'pharmacological synthesis' of the testosterone action.

It has often been argued that if a crude hormone mixture can be split into two fractions whose pharmacological actions are qualitatively different, one may conclude that the hormone mixture contains at least two chemically distinct active compounds. This argument, which is being used especially in connection with fractionation of pituitary extracts, is invalid in the light of our observations. We note that two concentrations of a hormone mixture in which the proportion of the active ingredients remains unchanged, may also have qualitatively different actions. Indeed, it has been emphasized (1, 2) that even two different concentrations of a single compound, such as testosterone, may have entirely different actions inasmuch as low doses damage, while high doses protect the seminiferous epithelium.

SUMMARY

Experiments in the rat show that a mixture containing a constant proportion of Δ^5-androstene-3(β),17(α)-diol and α-estradiol causes more pronounced testis atrophy in small than in large doses. Thus it imitates the effect of testosterone. Estradiol given by itself causes testis atrophy at all effective dosage levels, while the androstenediol has no such effect, whatever the quantity administered. The experiments are regarded as support for the concept that it is the folliculoid action of the testosterone molecule which is responsible for its anti-spermatogenic effect, and that by adding estradiol to the androstenediol, which in itself is poor in folliculoid potency, we obtain a mixture which in this respect imitates the effect of testosterone.

This investigation was performed with the aid of a grant received from the DesBergers-Bismol Laboratories of Montreal, Quebec. The author is also indebted to Dr. E. Schwenk of the Shering Corp., Bloomfield, N. J., for supplying the estradiol and androstenediol.

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ASSOCIATION NOTICE

ANNOUNCEMENT

EDITORIAL CHANGES, ENDOCRINOLOGY

Dr. E. B. Astwood and Dr. E. W. Dempsey, of the Harvard Medical School, have been appointed Managing Editor and Associate Managing Editor, respectively.

They will have the collaboration of a newly established Editorial Board consisting of Drs. J. S. L. Browne, E. T. Engle, C. G. Hartman, E. C. Kendall, F. C. Koch, C. N. H. Long and H. B. van Dyke.

The changes will become effective with the February issue.

NOTICE

UPON ADVICE of the Office of Defense Transportation, the meeting of the Association for the Study of Internal Secretions, tentatively scheduled for April 5th and 6th, 1943 at Cleveland, Ohio, is indefinitely postponed.

E. K. SHELTON, *President*

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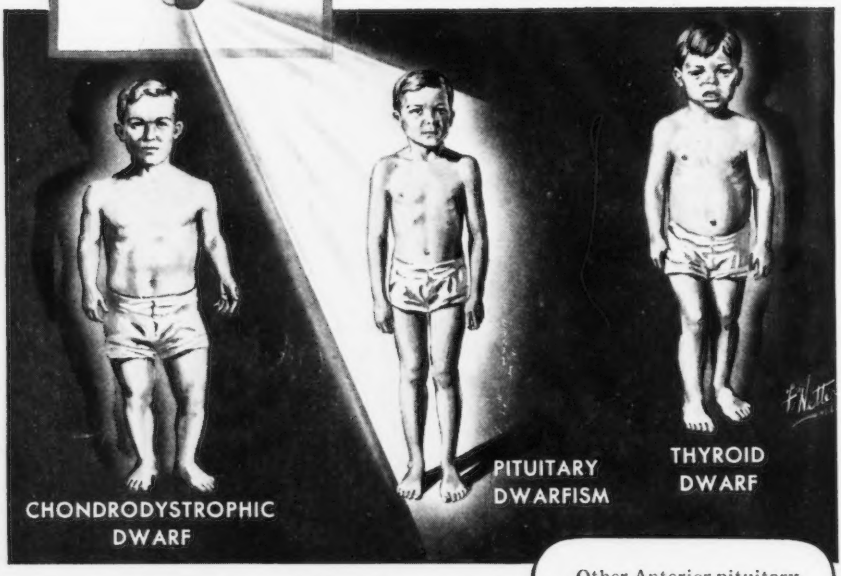

● True dwarfism is relatively rare, but the clinical problem of deficient growth of some degree is commonly encountered. Inadequate function of the pituitary gland is often basically responsible for this condition. Many of the cases show other evidences of pituitary hypofunction such as hypogenitalism.

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